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BIOLOGICAL EXTRACTION AND ACCUMULATIONS IN STREAMS

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THE UNIVERSITY OF MICHIGAN
DEPARTMENT OF ENVIRONMENTAL HEALTH
School of Public Health

Final Report

BIOLOGICAL EXTRACTION AND ACCUMULATIONS IN STREAMS

Covering Period June 1, 1966 - May 31, 1968

Principal Investigators:

June 1, 1962 - December 31, 1966 - C. J. Velz
January 1, 1967 - May 31, 1968 - John J. Gannon

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SECTION I

INTRODUCTION

The main objective of this the study has been were an investigation of the phenomena of biological extraction and accumulation in streams in terms of its influence on river self-purification, particularly as it related to oxygen balance. The original research proposal listed more precisely the following three primary aims:

1. To determine the basic underlying environmental conditions which give rise to formations of filamentous growths in streams.

2. To determine the mode of action and factors—biologic, hydrologic hydraulic—governing the rate of extraction and accumulation of BOD.

3. To investigate means of eliminating or controlling such formation and growth in streams.

In order to accomplish those objectives, the research effort has been organized into three integrated phases: (1) Laboratory investigations, (2) studies in channels, and (3) studies in natural streams. A progress report covering the period June 1, 1962—June 1, 1966 was prepared and submitted to the sponsor with a brief summary of the findings during this period presented as part of this introduction. Professor G. M. Ridenour retired in June, 1966, and Professor C. J. Velz, the principal investigator of the project, retired on December 31, 1966. Originally the study was scheduled for completion on May 31, 1967, but it was extended until May 31, 1968 without additional funds to allow certain parts of the investigation to continue, and to provide support for graduate students working on these studies.

A section of this report deals with *Sphaerotilus* cultural requirements under dynamic flow conditions prepared by Edward Armbruster, and a second section deals with the biochemical components of the sheath of *Sphaerotilus natans* by Farouq Shafie. Both of the studies are part of the laboratory investigations phase of the project.

Section IV dealing with the ecological relationships of *Sphaerotilus* and associated organisms under continuous flow conditions by John D. Phaup, and Section V covering reaeration and photosynthetic oxygen relationships in experimental channels by Peter Meier and Ronald Sharpin both deal with the experimental channel phase of the project.

For the convenience of the reader who does not have access to the earlier progress reports, a brief summary of the activities and findings of the project for the period June 1, 1962 to June 1, 1966 taken directly from a progress report for this period is presented as follows:

General Summary

June 1, 1962 - June 1966

The results of studies in the various phases of this investigation are as yet far from giving any decisive answer to the proposed objectives set forth in the introduction. Due to the obstinacy of the Sphaerotilus organism in yielding any well defined answers as to its specific nutritional needs and biochemical pathways of metabolism, its ecological relationships to associated organisms and its pleomorphic characteristics. The results to date with a few exceptions, have been largely indicative only in their nature. However, much has been learned as to feasible techniques that can be followed in the various areas of the investigation and future lines of study that need to be emphasized or repeated in order to reach more definite conclusions.

Each phase, namely Phase I (Laboratory Studies), Phase II (Outdoor Experimental Channel), and Phase III (Studies in Natural Streams) is sumarized as follows:

Phase I

The earlier part of the laboratory studies were spent in becoming familiar with techniques needed for the isolation of the organism from natural sources and its culture in the laboratory. This was succeeded by investigation of the physical conditions and nutritional needs of the different isolates which would permit greatest biomass accumulation and some method of measuring the biomass formed. Following this, studies were conducted on oxygen utilization rates of Sphaerotilus in comparison with natural mixed flora under different laboratory condition using both artificial and natural basal substrates. Minimum nutritional requirements of the various isolates were also investigated and their comparative response to similar conditions of cultural environment. Other facets examined were concerned with the extent of extracellular absorption of organic material by the organism such as glucose as compared to metabolic utilization of the same material.

In all of these laboratory studies the researchers on this project have found the greatest handicap lies in the lack of any method for identifying possible differences between subspecies or strains of the so-called Sphaerotilus organism. There is also even some disagreememt among other researchers as to possible identification of different genera of the sheathed bacteria. This whole issue is made all the more complex by the pleomorphic characteristics of the organism in response to different nutritional conditions and time of laboratory culture. As a result of this situation, a major effort has been made and is continuing on studies employing the use of fluorescent-antibody techniques for characterization of the organism. As indicated in the result of studies to date in one of the following sections this technique seems to show considerable promise. Accompanied by continued simultaneous studies of nutritional characteristics and requirements of different isolates along similar lines to those conducted in the past on this project, considerable clarification of the present confusion should result.

As previously indicated, other sections of the laboratory studies have been completed but the results were more indicative than conslusive. More extensive investigations should continue on rates of biochemical oxygen demand of different areas of a Sphaerotilus infested stream operating under natural conditions of pollution and decomposition. This is needed as a check on laboratory studies completed under this project which indicate that the life cycle of Sphaerotilus in the stream from initial growth to lysis and decay may affect the rate of oxygen demand to an abnormal extent.

Phase II

The primary purpose of the channel phase is the measurement under dynamic conditions of the rate of extraction and accumulation of BOD by biological mass, and the resulting oxygen balance using an experimental channel. The primary objective involved the design, fabrication and assembly of a suitable outdoor channel, which had flexibility in both length and cross section. The first assembly (approximately 200 feet in length) was constructed and erected at the site of the Ann Arbor sewage treatment plant adjacent to the Huron River during the summer of 1964. Modifications in design including bend sections allowed a reassembly in the summer of 1965 using the complete 640 feet.

Experimental objectives during 1964 and 1965 included the evaluation of the hydraulic characteristics under a range of flows, continuous monitoring of channel water quality and limited reaeration evaluation, and development of slime growth in the channel including measurement of BOD and D.O. under varying nutrient, flow, velocity, and temperature conditions.

Where continuous monitoring of dissolved oxygen was performed, it was seen that a typical diurnal curve developed with the peak reached between 12:00 noon and 3:00 p.m. and the minimum in the early morning hours. The limited reaeration studies involved thirteen runs, including two night runs, using sodium sulfite as the deoxygenating agent with a cobalt catalyst. Oxygen increase in the range of 1 to 2 mg/l was observed between the beginning and end of the channel under the experimental conditions followed.

During the 1965 test period eight different experiments ranging from four to nine days in length, were conducted measuring BOD and D.O. and relating these measurements to slime growth. In Experiment A it was demonstrated that BOD reduction reaches a maximum level during "saturation population" conditions which follows the biological growth weight increase as measured by attachment strings, and then the BOD reduction decreases in magnitude as the detached slime is picked up in samples collected at the end of the channel. During a period of maximum BOD reduction, the channel k_c reached a high level of 54.

In addition, extensive biological investigations were initiated in 1965, first for the purpose of developing procedures which would permit reliable quantitation of growth under simulated stream conditions, then to determine character of the growth, and lastly to evaluate the role of associated organisms in stimulating or inhibiting the development of a Sphaerotilus bloom.

Sphaerotilus was stimulated to grow in the channel by the addition of sucrose to incoming river water. A series of strings were suspended at several points to serve as attachment sites. These strings were removed at 24-hour intervals and the dry weight of attached growth determined in an attempt at quantitation. Attached organisms were removed from a measured length of string, identified and enumerated at 24-hour intervals to determine changes in the population following introduction of nutrient and growth of Sphaerotilus. Plankton determinations were made on influent river water to serve as a base line.

Under conditions of maximum growth an equilibrium is reached such that the amount of material sloughing off and dispersing throughout the channel equals or exceeds the amount of new material formed. The term "saturation population" is proposed to describe this condition. Under "saturation population" conditions, the interior of the floc is blackened with an odor of hydrogen sulfide present and anaerobic conditions prevail in all but the uppermost layers of cells. This indicates that there is critical thickness of the slime mass, after which it is no longer permeable to diffusion of oxygen and/or other gases.

It appears that the magnitude of growth is directly proportional to both temperature and velocity over the range tested.

Phase III

Analyses of river survey data on streams in which infestation of Sphaerotilus has occurred continues with the primary objective of developing relations between hydraulic parameters and BOD extraction and removal rates. Effort has centered this year on two rivers, the Paw Paw in Michigan and the Jackson-James in Virginia.

A unique opportunity for a longitudinal study in time is afforded on the Jackson-James to evaluate changes associated with two dramatic alterations in conditions, namely, biological treatment of major wastes and low flow augmentation.

The principal investigator made a detailed study and report on the reach below Covington, Virginia in 1953 before treatment, at which time Sphaerotilus infestation predominated with a BOD removal rate of about 0.4. On the basis of that study, the West Virginia Pulp and Paper Co. constructed the biological treatment system.

Since operation of the treatment works the company carries out routine river and waste effluent surveys almost daily except on weekends. Arrangements have been made, through the courtesy of the company, to make available these extensive data for restudy. From a visual survey of the river, a marked difference between the "before" and the "after" treatment condition is evident. Two years of routine "after" river sampling data are now being analyzed to determine if biological extraction and accumulation has been eliminated and self-purification has reverted to normal rate of BOD satisfaction in transit along the course, or if Sphaerotilus conditions persist, and if so, what effect treatment has had upon the rate of BOD removal. It is anticipated that two additional years of river survey data will also be made available. While runoff of the Jackson-James is extremely flashy, it is anticipated that stable hydrologic periods will be reflected among the routine daily river sampling data.

In the 1953 report an evaluation of the benefit of the proposed Gathright-Falling Spring low flow regulation reservoir was made. However, this development did not receive Congressional appropriation and was not undertaken. It now appears that the reservoir development, in some form, will be constructed.

The natural drought severity at Covington, expected on the average in the long run once in ten years, is 70 cfs as a consecutive 7-day average. The original Gathright development (with regulation to balance out hydro power pulsations) provided storage to assure about 500 cfs at Covington. It is now proposed to continue intensive study of the Jackson-James to evaluate the effect of the proposed low flow regulation upon biological extraction with particular emphasis upon opportunity for accumulation. Present analyses under natural runoff indicate potentiality of elimination of accumulation opportunity if reservoir releases were employed to maintain, or periodically provide, scouring velocities as insurance against ever attaining equilibrium accumulation.

The availability of the extensive survey data on the Jackson-James constitutes an invaluable contribution to the research study.

SECTION II

SPHAEROTILUS CULTURAL REQUIREMENTS UNDER DYNAMIC FLOW CONDITIONS

by

Edward H. Armbruster

Lecturer in Public Health Engineering

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INTRODUCTION

The data reported herein covers the period of June 1, 1966 to June 1, 1967 and are presented to supplement the data of the previous three reports for the period of June 1, 1962 to June 1, 1966. These current studies can be described under four headings.

1. Utilization of organic carbon compounds
2. Utilization of nitrogen compounds
3. Growth factor stimulation
4. Growth inhibition studies

Multiple isolates of Sphaerotilus were used in all of the above studies as a check for strain differences and all results were confirmed by at least one repeat trial before the data was considered valid and included in the report.

MATERIALS AND METHODS

Laboratory channels which were used so successfully in the previously reported study of the inorganic chemical requirements of Sphaerotilus are described as a matter of convenience to the reader. In addition, photographs of these channels are shown in Figures 1 and 2.

Six pieces of 1" thick pine board were cut to 9" by 24" rectangles. Eight steps 3" long and 3/4" in height were cut into these sections. Each step then had a 3" section of quarterround nailed to it to provide a cradle for a 50 ml polycarbonate centrifuge tube. The 4" centrifuge tubes were cut into two pieces lengthwise slightly above the center and the deeper half used in the experiments. The six cascading channel supports were then mounted to a wood base. A 1" x 1" wood strip was fastened under the base at the discharge end so as to provide a pitch of 1" in 24" and thus create pools in each of the centrifuge tubes mounted on the steps. A drain trough was attached to the base to carry off the discharge from each of the six channels. The total volume of each pool was approximately 10 ml so that an entire channel held only 80 ml. The nutrient solution was introduced into the upper pool at a rate of 500-600 ml per hour, which theoretically provided 6-7 changes of medium per hour.

A pipette tip, inserted into rubber tubing from the nutrient reservoir, permitted dropwise introduction of the feed solution to the upper pools of each channel. Each channel had a separate 5 gallon Pyrex aspirater bottle to serve as a reservoir of the feed solution. The rate of solution feed to the channels was

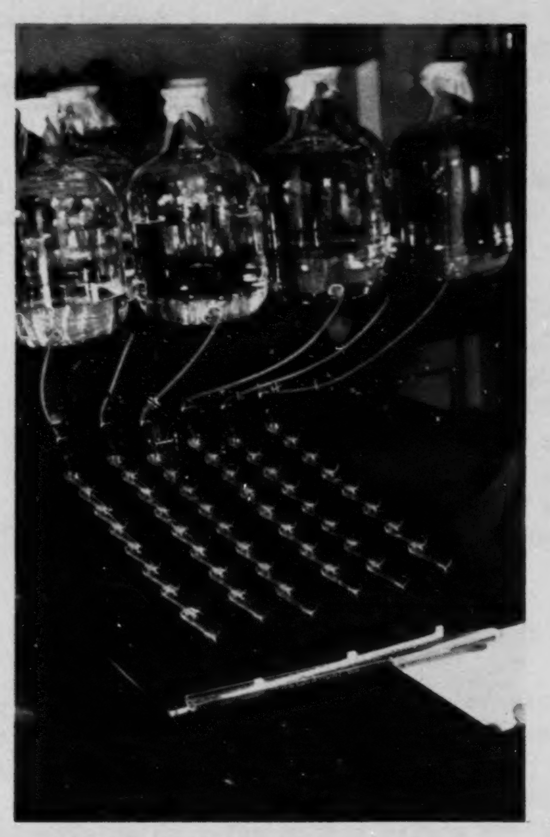

Figure 1. Assembly of cascading flumes and media feeding devices.

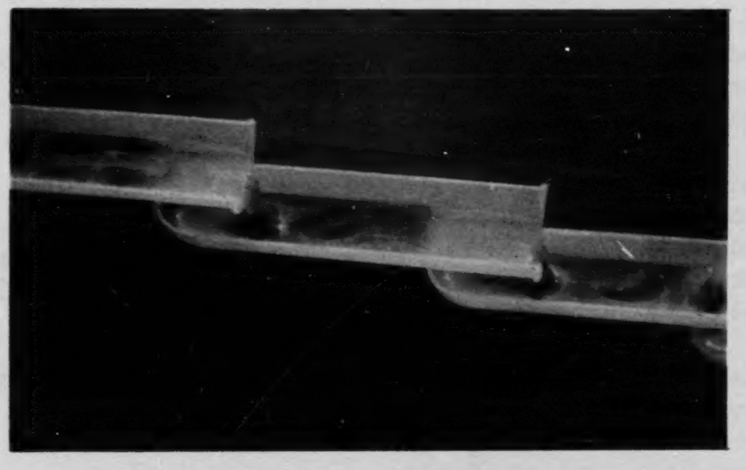

Figure 2. A section of one of the above flumes showing amount of *Sphaerotilus* growth.

adjusted by means of a simple screw clamp. A constant head device consisting of a length of glass tubing inserted through the rubber stopper of the aspirater bottle and reaching to within an inch of the bottom of the bottle held the rate of feed constant.

The basal medium consisting of a few inorganic salts and a utilizable carbon source in dilute solution provided all the nutrients necessary for growth under dynamic conditions. The medium adopted for these studies was as follows:

NH_4Cl		1.7 mg
KH_2PO_4		8.5 mg
K_2HPO_4		21.5 mg
Na_2HPO_4	.7 H_2O	34.4 mg
$MgSO_4$	.7 H_2O	22.5 mg
$CaCl_2$		27.5 mg
$FeCl_3$	.6 H_2O	0.25 mg
Carbon source		8.9×10^{-4} molar
H_2O		1000 ml

pH adjusted to 7.1 ± 0.1 with NaOH where necessary

The various nutrients solutions were not sterilized, partly to eliminate breakdown of the substrates by autoclaving and partly due to the difficulties encountered with the handling of the volumes. It was found that contamination could be avoided by daily washing of the reservoir carboys and tubing in 180°F water and sterilizing the entire system prior to an experiment. Fresh nutrient solutions were prepared daily.

Four to six pellicles of the test culture, grown in trypticase glycerol broth, were suspended in 50 ml of BOD dilution water. A Waring Blendor, with a sterile low volume blendor jar, was used to prepare the seeding suspension. Five ml of the homogeneous suspension was added to the top pool of the channels after the channels had been filled with the nutrient solution. A very low rate of nutrient feed was utilized for the first two hours after seeding. At this time the desired feed rate was set.

After 72 hours, the growth was removed from each channel with the aid of a rubber policeman and placed in a centrifuge tube. After centrifuging, the free water was poured off and the biomass placed into an aluminum foil planchet for drying and weighing, thus permitting comparisons of dry weight of Sphaerotilus biomass produced under various conditions.

Despite the fact that completely aseptic techniques were not feasible, contamination was not a factor in the accumulated data since parameter of measurement and comparison was the dry weight of biomass produced and this biomass was consistently better than 99% Sphaerotilus as determined microscopically.

EXPERIMENTAL RESULTS

UTILIZATION OF ORGANIC CARBON COMPOUNDS

The literature on utilization of carbon compounds by Sphaerotilus contains a number of conflicting reports. Some possible explanations for these discrepancies are:

(1) The relatively high concentrations of compound necessitated in batch culture procedures,
(2) the nutrient basal media contained other carbon compounds in addition to one under study, and
(3) the possibility of breakdown of the material during sterilization.

Therefore it was decided to restudy a number of carbon compounds using the previously described laboratory channels which permitted the growth of Sphaerotilus under dynamic and continuous flow conditions more closely approximating the natural habitat of the organisms than the oft used batch culture systems.

In these tests, a large variety of carbon compounds were compared individually against sodium lactate of the basal medium. The concentration of the compounds was 8.9×10^{-4} molar (except where noted) which was the molarity of 100 mg/l. of the sodium lactate. These media were prepared fresh daily in 20 liter volumes. A feed rate of 10 ml per minute was used in these studies.

With each series of tests, one flume contained sodium lactate to serve as a control on organism variation between tests. For comparability, the results of these tests are expressed as percentage of growth of the control. This was necessary due to temperature changes over the period of testing, a factor which could not be rigidly controlled due to the unique set-up of the flumes. A second factor which influenced total weights was periodic replacement of the plastic channel pools which tended to warp out of shape during sterilization between runs. Consequently the pool volumes of the channels had some variation which influenced biomass produced between the weekly runs, but not within a test series. The average dry weights of the biomass controls were 70,90, and 70 milligrams for isolates 1064, 365 and 665, respectively. The results expressed in Table I are an average value of at least two trials. If any appreciable variation occurred between the two trials for a compound, the results are expressed as a range.

From these data it can readily be seen that a number of organic compounds will serve as sole carbon sources for Sphaerotilus. Seventeen of nineteen tested were able to be utilized by isolate 365. Unfortunately isolate 665 was lost while being carried in stock culture before all tests were completed for this organism. Of all the compounds tested, all test strains produced the greatest biomass with pyruvic, malic, and lactic acids. Butyric, citric and acetic acids were also utilized to an appreciable extent. Maleic, propionic, and formic acids were able to be utilized, but only slightly. Isobutyric acid was the only acid tested which was unable to support any growth.

TABLE I

DRY WEIGHT OF SPHAEROTILUS BIOMASS PRODUCED AS % OF LACTATE CONTROLS FOR VARIOUS COMPOUND AS SOLE CARBON SOURCES

	1064	365	665
Formic acid	7	6	0
Acetic acid	50	48	
Propionic acid	15	8	1
Pyruvic acid	117	110	114
Malic acid	101	83	134
Maleic acid	18	13	1
Butyric acid	95	57	
Isobutyric acid	0	0	
Citric acid	72	35	63
Ethyl alcohol	88	0	0
Isopropyl alcohol	0	0	0
Glycerol	91	46	
Dextrose	90	2 - 50	2
Sucrose	49	0 - 50	0 - 54
Maltose	87	6 - 18	2
Levulose	135	0 - 2	0
Lactose	10	0	0
Dextrin	82	55	
Na Lactate (control)	100 (70 mg)	100 (90 mg)	100 (70 mg)

Note: Citric acid, ethyl alcohol, isopropyl alcohol - 100 mg/l
All other compounds are 8.9×10^{-4} molar.

Significantly the common conception that carbohydrates alone as a carbon source stimulate Sphaerotilus is not born out by these test results. Only strain 1064 was able to use all the carbohydrates tested with yields ranging from moderate to excellent except for lactose which was slight. The other two test strains did not show correlation with isolate 1064 and even demonstrated inconsistency in results between replicate trials. Another example of variation between isolates is demonstrated with ethyl alcohol as the carbon source. Good growth was obtained with isolate 1064, while neither isolate 365 nor 665 could utilize this carbon source to any degree.

With these marked differences in growth results manifest among the three test strains employed, it is to be expected that differences among test strains of other workers are very likely and may be a major factor in the conflicting results reported in the literature. Also, the fact that most of the investigations reported in the literature have used different and richer basal media quite probably magnifies the variations due to the fact that more building blocks are present for adaptive enzyme systems.

It may be concluded from these results that a number of organic carbon compounds will serve as the sole carbon source for Sphaerotilus in a flowing system, but that marked differences can be expected among strains.

UTILIZATION OF NITROGEN COMPOUNDS

Previously reported studies of this project using the laboratory flumes have shown Sphaerotilus can utilize either NH_3 or NO_3 as sole sources of nitrogen. The ability of Sphaerotilus to utilize organic nitrogen as the sole source, however, has been uncertain, as reflected by the conflicting results reported in the literature. Two factors may explain, in part, differences among earlier work: the basal media employed may have contained peptones or other nitrogenous compounds in addition to the one under investigation, or the Sphaerotilus may have utilized the carbonaceous portion of the molecule of the nitrogenous compound.

Accordingly, two series of experiments have been conducted in which 16 organic nitrogen compounds have been tested as to ability to serve as the sole source, and further to explore the possibility of their acting both as a carbon and a nitrogen source.

In these experiments the Lactate BOD water basal medium was modified to eliminate NH_3 by treating the distilled water with Folin's Permutit resin and by deleting the NH_4Cl. The lactate concentration of the organic nitrogen sources, mainly amino acids, was 125 mgm/l. After addition of the nitrogen sources pH was restored to 7 by addition of NaOH. One hundred ml. volumes of the various media were placed in 150 ml Ehrlenmeyer flasks with Morton stainless steel closures to eliminate contamination by wisps of cotton. The solutions were sterilized by autoclaving at 10 lb pressure for 10 minutes.

Three different isolates of Sphaerotilus carried in the trypticase glycerol medium were used for seeding. A pellicle from a seven day tube culture was transferred to a sterile screw capped test tube containing 5 ml of sterile water and several glass beads. The pellicle was dispersed by vigorous shaking and two loops of the resulting suspension were used to seed the test flasks. The flasks were incubated at 27°C for four days, at which time they were checked for growth. Microscopic examination of the seeding material revealed that the cells contained a large number of inclusion bodies, which possibly could function as reserve nitrogen sources for the organisms. In order to minimize this potential influence on the test results, subcultures were made to fresh flasks of test media after the 4 days incubation of the primary flasks. The subcultures were then also incubated for four days at 27°C prior to reading. Table II represents the results based on visual estimation of the amounts of growth.

TABLE II

UTILIZATION OF VARIOUS ORGANIC NITROGEN COMPOUNDS BY SPHAERmax SPHAERO

	Sphaerotilus Isolate					
	Strain$_{365}$		Strain$_{665}$		Strain$_{1064}$	
	Primary	Subculture	Primary	Subculture	Primary	Subculture
Control	+	+	+	+	+	+
1-Alanine	3+	2+	+	2+	+	+
1-Arginine	4+	4+	4+	3+	3+	3+
1-Asparagine	4+	3+	2+	2+	2+	2+
1-Aspartic Acid	+	+	+	+	-	-
1-Glycine	4+	4+	3+	4+	2+	2+
1-Leucine	2+	2+	+	2+	+	+
Methionine	2+	2+	+	+	+	3+
1-Phenylalanine	3+	4+	2+	3+	+	2+
1-Proline	2+	+	4+	3+	4+	4+
Pyridoxine hydrochloride	+	+	+	-	+	+
1-Serine	3+	3+	2+	3+	2+	2+
1-Threonine	3+	3+	2+	2+	3+	3+
1-Tryptophane	+	4+	3+	4+	3+	4+
1-Tyrosine	3+	4+	3+	4+	3+	4+
1-Valine	3+	4+	2+	3+	4+	3+
Vitamin B$_{12}$	3+	2+	2+	+	2+	2+

CODE: - = no growth
+ = scant, 1-5 filaments
2+ = light growth
3+ = moderate
4+ = heavy growth

The traces of growth in the control flasks were attributed to small amounts of NH$_3$ reabsorbed into the medium after sterilization and verified by Nesslers reagent. Therefore, a growth reaction greater than 1 + was required to show organic nitrogen utilization. Arginine, Tryptophane, Tyrosine, and Valine provide good readily usable nitrogen sources for all three strains of Sphaerotilus as measured by ability of the organisms to reproduce. Asparagine, Phenylalanine, Serine and Threonine were also utilizable as nitrogen sources, but to a lesser extent. Variable results were evident with Alanine, Leucine, Methionine, Proline and Vitamine B$_{12}$. Further studies are needed to determine whether this is due to species or strain differences, or to the ability to produce adaptive enzymes rapidly. In several instances, most notably with Tryptophane, the subcultures produced greater growth indicating development of an adaptive enzyme system.

The second series of tests was oriented to determination if these compounds could serve as both carbon and nitrogen sources for Sphaerotilus. The procedure was identical to that for the first series with the sole exception that the sodium lactate was deleted from the basal medium thus eliminating the organic carbon. These results are given in Table III.

TABLE III

UTILIZATION OF VARIOUS ORGANIC NITROGEN COMPOUNDS BY SPHAEROTILUS IN NITROGEN AND CARBON LIMITING MEDIUM

	Primary	Subculture	Primary	Subculture	Primary	Subculture
Control	-	-	-	-	-	-
1-Alanine	+	2+	+	2+	+	2+
1-Arginine	+	+	+	-	+	-
1-Asparagine	3+	+	+	+	+	+
1-Aspartic Acid	+	-	+	-	-	-
1-Glycine	+	+	+	-	+	+
1-Leucine	+	+	+	++	-	-
Methionine	+	-	+	-	+	-
1-Phenylalanine	-	-	+	2+	-	-
1-Proline	3+	3+	2+	2+	4+	3+
Pyridoxine hydrochloride	+	+	-	-	+	-
1-Serine	-	-	-	-	-	-
1-Threonine	+	+	-	-	+	+
1-Tryptophane	+	+	+	3+	-	-
1-Tyrosine	2+	2+	2+	3+	2+	2+
1-Valine	+	-	+	-	+	-
Vitamin B_{12}	-	-	-	-	-	-

CODE: - = no growth
+ = scant, 1-5 filaments
2+ = light growth
3+ = moderate growth
4+ = heavy growth

No growth resulted in the controls which contained no organic carbon sources. A number of the organic compounds were able to support slight (1+) growth in the primary flasks and several in the secondary flasks. The growth in the primary tubes may be attributed to stored food reserves in the numerous inclusion bodies of the seed organisms. The 1+ growth in the secondary was quite variable for the three strains of Sphaerotilus used. With traces of ammonia absorbed in the media, the growth is indicative of utilization of the carbonaceous portion of the molecule; however, further studies on this point are warranted.

Proline, and to a lesser extent, Tyrosine and Alanine demonstrated that they could serve as both carbon and nitrogen sources for the three test strains. Serine and Vitamine B_{12} by themselves were not able to support growth.

Conclusions

As with the previous carbohydrate studies on Sphaerotilus, variation among strains is also evident in the utilization of the various organic nitrogen compounds. In the presence of a good carbon source, (sodium lactate), the best results were obtained with Arginine, Tryptophane, Tyrosine, and Valine as the organic nitrogen source. In the absence of a carbon source, Tyrosine and Proline gave the best growth.

GROWTH FACTOR STIMULATION

Flume studies have shown that Sphaerotilus has very simple chemical requirements for growth. Either NH_3 or NO_3 will suffice as sole nitrogen sources as well as some amino acids. A wide variety of carbon compounds ranging from ethyl alcohol or acetic acid up to dextrins will function as sole carbon sources under the flowing conditions used in the laboratory test flumes. Both PO_4 and Mg ions are required for growth and the presence of Ca is indicated necessary for the typical sheath production so characteristic of this genus. With such simple nutrient requirements, Sphaerotilus would be expected to be found in nearly all flowing surface waters.

A number of water samples have been taken from streams, county drains and ditches in southeastern Michigan. Sphaerotilus has been cultured from all samples taken with test volumes ranging from 10 - 15 ml. The isolation procedure was that reported previously in which the Lactate - BOD water medium was used. These waters had no known previous history of Sphaerotilus infestations, nor was there any visual evidence of Sphaerotilus present during the sampling period.

Sphaerotilus is apparently a part of the normal flora of these streams and probably many other streams in the United States. However infestations or blooms are rarely, if ever, present in these streams despite the fact that sufficient nutrients are usually present to support the organisms. Growth stimulants as suggested by Waitz and Lackey[1] may be one of the factors necessary to produce these blooms. It is quite within the realm of speculation that the growth stimulants are supplied in wood pulping and certain types of cannery wastes which permit Sphaerotilus to become the predominant type of growth. In the absence of the growth stimulants, the other flora predominate and utilize the nutrient material more efficiently, thus suppressing the Sphaerotilus growth and hence preventing the characteristic infestation by which these sheathed bacteria are recognized in streams.

Vitamin B_{12} (cyanocobalamine) was found by Okrend and Dondero[2] to promote Sphaerotilus growth in batch cultures. Therefore this compound was selected to be used in the laboratory channels to measure its stimulatory effect under conditions more closely approximating those of streams.

The same basic procedure was followed as in the previous channel studies using the Lactate - BOD water medium. Channels were set up with 0, 5, and 10 ug/l of B_{12} added to the basal medium. A channel containing only 10 ug/l of B_{12} in BOD waters served as a control. This experiment was repeated twice with each of the two test strains, 365 and 1064. Table IV presents the data expressed as dry weights of Sphaerotilus obtained after 72 hours growth in the channels.

TABLE IV

STIMULATORY EFFECT OF VITAMIN B_{12} ON SPHAEROTILUS

	(365)		(1064)	
	Trial 1	Trial 2	Trial 1	Trial 2
Control - No B_{12}	89	94	100	96
5 ug/l B_{12}	145	140	159	150
10 ug/l B_{12}	156	156	168	159
B_{12} in BOD water	0	0	0	0

NOTE: All values are expressed as dry weights in mgms.

It can be seen from these data that vitamin B_{12} by itself did not serve as a nutrient in this experiment. The basal lactate medium containing no B_{12} supported Sphaerotilus biomass equivalent to 92 and 98 milligrams as dry weights for the two strains tested, indicating the presence of this material in a basal medium is not essential for growth.

However when the B_{12} was added to the lactate basal medium at a concentration of 10 ug/l, the average dry weight yield of Sphaerotilus increased by 70% for isolate 365 and 67% for isolate 1064. Even 5 ug/l of B_{12} increased the yield by 55% and 59% respectively for these two isolates. Another way to view the data is that for additions of less than 0.5 milligrams or 0.25 milligrams of B_{12} to the channels, over a period of 3 days, increased the dry weight biomass yields by approximately 65 milligrams and 55 milligrams respectively. This relatively large increase in weight in the presence of trace amounts of vitamin B_{12} definitely shows a stimulatory effect.

Another observation of note was that the lag phase appeared to be shortened by 30% as judged by a visual appearance of the growth in the channels. Microscopic examinations of growth demonstrated that contamination was slight since the biomass consisted of 99.9% or better Sphaerotilus organisms and sheath material.

Other members of the water soluable vitamin B group are well-known for their ability to function as growth factors for various organisms. To date, two of these compounds, thiamine hydrochloride and biotin, have been investigated using the procedure described for vitamin B_{12}.

The basal medium was supplemented with 10 and 100 ugs/l of thiamine hydrochloride and 82 and 87 milligrams of biomass dry weight respectively were produced against 90 milligrams for the control with isolate 365. The biotin was added in 5 and 10 ugs/l amounts and 101 and 99 milligrams of biomass resulted as compared to the control of 99 milligrams.

In each case the results are expressed as the mean of two trials. As determined by the dry weight of biomass produced, there is no evidence of stimulatory effect for either thiamine hydrochloride or biotin.

GROWTH INHIBITION STUDIES

One of the problems still confronting stream biologists is the control of Sphaerotilus infestations in streams which at times may create a nuisance over a distance measured in miles. To date, no practical solution for effective control of this growth has been developed. One of the problems hampering this phase of the research is the lack of simple laboratory procedures simulating the natural environment of these slime-producing organisms using low volumes of substrate and a simple method of quantitating the results of the procedure.

The series of laboratory channels, constructed with plastic test tubes, have proven valuable for the measurement of the nutrient requirements of Sphaerotilus under dynamic and continuous flow conditions. Measurement of dry weight of the biomass produced was reproducible and permitted quantitative comparisons of substrates. Therefore, it appears possible that these channels, which more closely simulate the natural environment of Sphaerotilus than the commonly used batch culture systems, could be utilized in quantitative studies on inhibition of the organism by chemical additives.

In order to test this assumption six channels were set up and seeded with isolates 1064, 365, and 6R in the manner described previously. The substrate used was 100 mg/l of sodium lactate in BOD dilution water. The inhibiting agent added to 3 of the channels was sodium chloride which was selected because field experiences have shown that Sphaerotilus growths are generally absent in tidal waters.

There are no measurable differences in growth for any of the isolates between the control channels and those containing as much as 3000 mg/l of sodium chloride in the basal medium. The results in the following table show a marked difference in biomass produced when this concentration was increased to 5000 mg/l, which incidently happens to be the amount of sodium chloride included in the formulations of numerous media used for bacterial culture.

TABLE V

INHIBITION OF SPHAEROTILUS BY 5000 mg/l OF NaCl

	mgm dry et. Biomass produced		
Isolate	Control	NaCl	% Inhibition
1064	77.4	40.4	48
365	72.4	27.5	62
6R	75.0	31.0	59

The inhibition of the Sphaerotilus growth ranged from 48-62% as measured by dry weights of biomass produced for the three different isolates used in this study. The growth in the three test channels did not resemble the controls since it no longer had the typical slime-like appearance and it tended to break up when disturbed. Microscopically, this was confirmed in that the cells were short rods occuring in relatively short distorted chains showing very little of occasional bits of sheath as opposed to the normal sheathed long chained Sphaerotilus in the controls. These results have shown that sodium chloride in a concentration frequently used to provide isotonic conditions for culture of most bacteria, is inhibiting to Sphaerotilus.

The next compound examined in this system was copper sulfate, which was selected since it is already used in control of aquatic growths. The channel set up remained unchanged and isolate 365 was seeded in the normal manner using the sodium lactate-BOD dilution water medium. Channels 1 and 2 served as controls, 3 and 4 had 0.25 mgm/l of copper sulfate added to the basal medium supply reservoir, and 5 and 6 had 0.5 mgm/l added to the basal medium. After 70 hours all growths were harvested. The results expressed as dry weight are given in the following table:

These results show that the inhibition of Sphaerotilus isolate 365 was virtually complete at a concentration of 0.5 mgm/l, and the growth was suppressed by greater than 50% during the 70 hour test in the presence of 0.25 mgm/l.

TABLE VI

INHIBITION OF ISOLATE 365 WITH COPPER SULFATE

Channel	mgm/1 $CuSO_4$	mgm growth dry weight
1	0	83.1
2	0	80.2
3	0.25	33.6
4	0.25	38.9
5	0.50	trace *
6	0.50	trace *

*The film of growth present appeared to be of a zoogleal nature with only rare Sphaerotilus filaments by microscopic examination.

These results show that the inhibition of Sphaerotilus isolate 365 was virtually complete at a concentration of 0.5 mgm/l, and the growth was suppressed by greater than 50% during the 70 hour test in the presence of 0.25 mgm/l.

However, the above results were typical of a situation in which Sphaerotilus was introduced as free cells and not as an attached biomass as would be the case in most field situations. Consequently, an experiment was set up introducing copper sulfate into the channels after the growth had produced a good attachment film throughout all the channels. All six channels were seeded as before and growth was permitted to become established for 24 hours. At this time, the Sphaerotilus biomass was harvested from two channels to determine the amount of growth present prior to the introduction of the copper sulfate. Two channels now has 0.5 mgm/l of copper sulfate added to the medium supply reservoirs. The remaining two received only the sodium lactate-BOD dilution water medium and thus served as untreated controls.

After 24 hours of treatment with copper sulfate, the growth of one of these channels was collected, along with a control. The final two channels were harvested after 48 hours from time of introduction of the copper sulfate. Additional experiments of this type followed in which longer treatment periods were used. The results typical of several trials with varying time periods are condensed in Table VII.

It can be seen from this data that the copper sulfate treatment of an established biomass is not as effective as was the treatment with free cells. In this case the inhibition of growth ranged from 41% to 49%. The results of the two types of experiments indicate thatthe copper sulfate at a feed rate of 0.5 mgm/l will not be able to completely eliminate a Sphaerotilus infestation, but it should prove of value in limiting the total biomass produced and probably the total area affected.

TABLE VII

INHIBITION OF ESTABLISHED GROWTH OF ISOLATE 365 BY 0.5 mgm/1 COPPER SULFATE

	Growth Prior to $CuSO_4$	24 hours treatment	48 hours treatment	96 hours treatment	194 hours treatment
$CuSO_4$	9	22	41	104	127
Control	9	39	81	175	214
% Inhibition	-	44%	49%	41%	41%

*Note: All figures in table are expressed as mgm dry weight of biomass harvested from channel.

A simple procedure has been presented which based on the results obtained with sodium chloride and copper sulfate will permit laboratory screening of compounds for control of Sphaerotilus infestations.

CONCLUSIONS

1. Seventeen of the nineteen carbon compounds tested were able to be utilized as sole carbon sources by Sphaerotilus. Pyruvic, malic, and lactic acid provided the best sources for all three test strains as measured by total biomass produced in three days.

2. Marked differences between strains and even between trials of one strain have been shown to exist.

3. The common belief that carbohydrates stimulate Sphaerotilus growth was not borne out since only one of the three test strains consistantly produced good yields of biomass with these substrates.

4. Tryosine and Proline were able to function as both carbon and nitrogen sources for all test strains.

5. In the presence of a good carbon source, Arginine, Tryptophane, Tyrosine, and Valine served as efficient nitrogen sources.

6. Vitamin B_{12} (cyanocobalamine) in amounts as low as 5 ug/l increased the biomass by more than 50% in the laboratory channels. No such effect was noted when thiamine hydrochloride was tested in concentrations up to 100 ug/l.

7. Copper sulfate as low as 0.25 mg/l suppressed the growth by better than 50% when tested against strains 365. A concentration of 0.5 mg permitted no visible growth of the organism, although an occasional filament could be observed microscopically.

8. Sodium chloride at a concentration of 5000 mg/l inhibited the growth from 48% - 62% for the three strains tested.

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SECTION III

THE BIOCHEMICAL COMPONENTS OF THE SHEATH OF *SPHAEROTILUS NATANS*

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INTRODUCTION

The voluminous literature written on Sphaerotilus deals primarily with aspects of water and sewage treatment, stream sanitation and the effects of varying physical and chemical parameters on crude cultures of this organism. In contrast, information on the nature of Sphaerotilus itself is mainly morphogenic derived from microscopic studies. This is primarily a result of the fact that this organism has received very little attention from the bacteriologist and the biochemist. Thus there yet remains much to be done of both practical and scientific interest. Little is known about the physiology and nutrition of Sphaerotilus: Synthetic, defined media, can be used for the cultivation of this organism but the amount of growth so obtained is very much less than that obtained from the inclusion of complex organic substances such as peptone and beef extract. The missing factors responsible for this large difference are not known. Another observation in this regard is that the effect of the added complex nutrients may vary, depending upon the other components of the culture media. A medium high in organic nitrogen and low in carbohydrate seems to give rise to cultures of predominantly swarming and slime cells instead of the "typical" cultures of insheathed chains of cells. As an explanation of this phenomenon, Dondero (1961) suggested the possibility that in rich medium, the cells are synthesized at a proportionately greater rate than is the sheath.

What are the specific factors and conditions which promote the formation of the sheath both in the pure culture flask and in the stream? How does the cell control the synthesis of this surface structure? These are still important unanswered questions and constitute the long term objective of this investigation. At this juncture it is felt that the logical and immediate concern of this project should be the definition of the biochemical components of the sheath, and the elucidation of their biological significance.

MATERIALS AND METHODS

As was stated above, our immediate concern in this investigation is the biochemical composition of the sheath and the significance of the various components. In order to do this it is first necessary to (1) obtain a pure typical culture of the organism (2) prepare the sheath material devoid of cellular elements; (3) establish criteria for purity of the sheath; and (4) develop assay procedures for the various chemical components involved.

F

ORGANISM AND CULTURAL CONDITIONS

The organism used in this study was isolated from the Huron River near Ann Arbor. It is identified as *Sphaerotilus natans* by morphological and cultural properties according to the description of Pringsheim (1949) and Stokes (1954). Cultures of this organism are grown in 250 ml Ehrlenmeyer flasks containing 80 ml of a synthetic, defined medium. This medium consists of 0.1 percent glucose, 0.02 percent NH_4Cl, 0.2 percent $MgSO_4 \cdot 7H_2O$, 0.005 percent $CaCl_2$, 0.0005 percent $FeCl_3 \cdot 6H_2O$, in 0.01 M phosphate buffer, pH 7.1. Cells for use in isolating sheath material are obtained by incubating flasks of inoculated medium at room temperature in stationary culture for four to five days. Cultures are harvested by centrifugation in a Servall Superspeed refrigerated centrifuge at 4500 x g for ten minutes.

ISOLATION OF THE SHEATH

Cells are prepared by washing three times in distilled water and Tris buffer, successively. The cells are then resuspended in Tris buffer, by homogenizing in a blender or tissue homogenizer at high speed for two to three minutes at zero degrees (to prevent contaminant growth). EDTA, lysozyme and sodium dodacyl sulfate are used in the order given to lyse the cells and remove cellular contents leaving intact sheath material as described by Romano *et al.*, (1963). The resulting sheath material is recovered by centrifugation and washed with buffer and distilled water until the washings do not foam when agitated indicating that the lytic agent had been removed. The preparations are then suspended in a minimal amount of distilled water, lyophilized and stored in a vacuum desiccator. Samples are removed as necessary for chemical analysis. To date, criteria for purity of the sheath material has been microscopic examination and DNA and RNA determinations. More definitive chemically criteria will probably have to await more complete chemical analysis of the isolated sheaths. One objective of this project will be the development of such chemical criteria of purity.

EXPERIMENTAL RESULTS

CHEMICAL ANALYSIS

Presence of Nucleic Acids

In an attempt to check the purity of the sheath material, analyses for the presence of DNA and RNA were carried out. Ten mg samples of the dry sheat material were defatted three times with 20 ml aliquots of chloroform-methanol (2:2 v/v) at room temperature for 30 minutes each. The samples were then dried, after separation from the solvent, in a vacuum desiccator, extracted three times with

5 ml each of 10 percent cold perchloric acid (PCA) and finally heated at 90°C for ten minutes. The resulting hydrolysate was filtered through fine cindered glass Buchner funnels. The hot and the cold PCA extracts were assayed by the Dische procedure for "purine" deoxyribose using anhydrous deoxyribose as standard. According to this procedure the DNA was estimated to constitute about ten percent of the sheath. The cold PCA extract contained no diphenylamine positive material. The PCA extracts were also scanned for absorption in the U.V. range and one peak was obtained at about 260 mμ, which is typical of DNA and RNA absorption. The diphenylamine procedure is not, however, a confirmatory test for the presence of DNA since other deoxysugars give a positive test. Thus a more direct approach was taken.

DNAse Treatment. This experiment was set up as follows:

Flask	Sheath	DNAse	Thymus DNA
1	30 mg	3 mg	None
2	30 mg	None	None
3	None	1 mg	1 mg
4	None	None	1 mg

All four flasks were put on the incubator shaker at 37°C for 18 hours. Three different controls were utilized. The DNAse used (Pancreatic DNAseI) was active, it increased the hyperchromicity of the pure DNA. The DNAse treatment did extract DNA from the sheath. One treatment with DNAse was sufficient to remove all the material absorbing at 260 mμ. This was demonstrated by two additional experiments: A) Retreatment of the sheath material for a second time with DNAse as was done before, and B) Extraction of the DNAse treated material with five percent hot PCA. There was no absorption at the 260 mμ wavelength after the second treatment with DNAse or hot PCA. This last experiment also demonstrated that there was no significant amount of RNA, since any RNA present cannot be broken down by the enzyme specific for DNA only, and therefore ought to show up if present in the hot PCA extract.

To demonstrate further the absence or presence of RNA, the sheath material was extracted with 1 N NaOH overnight at room temperature. The resulting hydrolysate was tested for "purine" ribose by the orcinol procedure (1947) with anhydrous ribose as standard. This test was positive, showing an RNA content of three percent. However, the color developed was of a different shade than that obtained with the standard reagent. Scanning of the colors showed the major peak to be at 430 mμ instead of 665 mμ as is the case with the standard ribose. Thus the usefulness of this test in the determination of RNA in the sheath material is questionable since there exists other interfering substances.

Moisture Content

The moisture content was determined gravimetrically on the lyophylized and vacuum **desiccator** stored sheath material. It was found to range from 8.7 to 9.28 percent. High moisture content is reported in the literature for glycoproteins (1966).

Carbohydrate Analysis

The carbohydrate analysis of the sheath can be summarized as follows:

Determinations	Percent Dry Weight	Method of Assay
Reducing sugars	36	Folin & Malmros (1929)
Neutral sugars	24	Roe (1955)
Hexosamines	12	Elson & Morgan (1933)

Thus the hexosamine content constitutes about one third of the carbohydrate content of the sheath.

IDENTIFICATION OF SUGARS

The sheath material was hydrolyzed in sealed tubes, in vacuo, with 2 N HCl for 15 hours. The resulting hydrolysate contained both the neutral and amino sugars which were separated from each other on Dowex 50 - X8(H^+) with distilled water as eluent for the neutral sugars and with acid, for the amino sugars. Both fractions were concentrated before and after desalting on Dowex-1(CO_3), in vacuo, to proper concentrations needed for paper chromatography. Whatman filter paper #40 (acid washed) was used for descending chromatography with four different solvent systems. After the chromatograms were dried the following reagents were used for the development of the spots:

(a) 0.5 ml saturated $AgNO_3$ in 100 ml of Acetone,
(b) 1.75 ml of saturated (19 N)NaOH in 100 ml 95 percent ethanol,
(c) 1 gm of sodium thiosulfate in 100 ml distilled water.

The above reagents were used in the order given. Glucanolactone, glucuronic acid, glucose, galactose, mannose, xylose, arabinose, and fucose were used as standards. The major spot obtained corresponded to glucose and there were two very faint spots that have not been identified as yet. The hexosamine was identified as glucosamine by the method of Salton (1958) which involved acetylation with acetic anhydride, and chromatography on Whatman #1 filter paper using methanol-water as the solvent system. Using this technique, identification of the amino sugar as glucosamine was definite since the differences in RF values of other amino sugars are quite large. A further testimony to the presence of only one amino sugar comes from the findings by ion exchange chromatography of only one peak which did not correspond to any of the known amino acids. This peak is reported in the literature to correspond to a hexosamine.

PROTEIN ANALYSIS

The protein content of the sheath material was found to be 30 percent. Determinations by the Lowry (1951) and the Biuret (Robinson and Hogden, 1940) methods were used and found to be in complete agreement.

Amino Acid Analysis

Amino acid analysis was determined using the automated ion exchange resin chromatography as described by Moore and Stein (1954). The amino acid composition in residues/100 residues was found to be:

Amino	Percent	Amino Acid	Percent
Cysteic Acid	7.4	Valine	4.5
Aspartic	4.9	Methionine	0.3
Threonine	3.9	Isoleucine	2.1
Serine	3.6	Leucine	4.3
Glutamic	6.2	Unknown Peak (Amino Sugar ?)	8.7
Proline	0.2	Tyrosine	29.6
Glycine	14.1	Lysine	5.9
Alanine	6.32	Histidine	0.7
1/2 Systine	1.4	Arginine	2.2

Two striking observations can be drawn from the above data: (1) Tyrosine comprises about 30 percent of the protein content of the sheath (i.e., one out of each three amino acid residues) or about ten percent of the whole sheath material. After searching the literature, we find, of all the proteins studied, none contains this much tyrosine. If all the amino acids mentioned above prove to be part of one protein then indeed it would be a unique protein, the function of which might have a bearing not only on the structure of the sheath but also on its function. (2) While there is a high content of tyrosine, no phenylalanine has been detected. One could ask what function does this tyrosine, have from a strictly structural sense in the sheath? Tyrosine, having a functional hydroxyl group, might be acting as the cross link, between a family of similar peptides and a skeleton of carbohydrates or as a bridging group between two skeletons—one made up of carbohydrates and the other of protein.

FRACTIONATION OF CHEMICAL COMPONENTS OF THE SHEATH

Methods for the separation of the sheath components must depend upon the types of bondings that exist between them. Preliminary indications are that the sheath may contain a glycoprotein or a mucopolysacharide. This means that there is no certain technique for separation and an extensive amount of arbitrary fractionation procedures will have to be used. These procedures will include treatment with reagents such as mild acid and alkalies, enzymatic hydrolysis by general proteolytic catalysts such as pronase and Nagarase and specific bond-splitting enzymes such as pepsin, trypsin, etc. The progress of digestion with each of such reagents will be monitored by (a) ultraviolet absorption spectra, (b) the ninhydrin method of Moore and Stein, (c) specific assays of known components such as neutral sugars, amino sugars and protein, and (d) different chromatographic procedures which would facilitate separation according to size, charge and other differences in the chemical structure.

So far we have found that a good portion of the sheath structure can be solubilized by treatment with dilute acid or alkali. Chromatography of these solubilized fractions on gel columns has indicated a strong chemical bond linking the polypeptide and the carbohydrate fraction, in that the two components move together. A smear was obtained on Bio-Gel-60 suggesting heterogenious size. Attempts to separate these fractions according to charge on DEAE cellulose columns, on the other hand, seems to give the consistent results. However, with this technique separation of some protein fractions from carbohydrate was obtained. More work will have to be done to ascertain whether the carbohydrate and protein are linked in all protein fractions or only in some.

SUMMARY AND CONCLUSIONS

The sheath from the organism *Sphaerotilus natans* was isolated as described by Romano *et al*., (1963). It was found to contain carbohydrates of relatively simple composition, protein of an unusual composition and a relatively high DNA content. The efforts of the author is continuing toward ascertaining the reality and interrelationship of these components in the sheath.

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SECTION IV

ECOLOGICAL RELATIONSHIPS OF SPHAEROTILUS AND ASSOCIATED ORGANISMS UNDER CONTINUOUS FLOW CONDITIONS

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INTRODUCTION

The profusion of literature concerning the biological effects of organic pollution was thoroughly reviewed by Hynes (1960). One consequence of the introduction of organic materials in some streams is the production of bacterial slime masses.

These massive colonies were collectively termed "sewage fungus" by the British (Butcher, 1932) which in the German literature is "abwasser-pilz" (Tiegs, 1938). These terms are misleading by virtue of the fact that most biological slimes are predominantly bacterial. Harrison and Heukelekian (1958) in their literature review proposed the term "slime infestation" to describe such growths, realizing that the slime organisms of the myxobacteriales or myxomycetes might be unjustly implicated. It seems too that the term "infestation" should be reserved for parasitological use. The term "biological floc" will be used here to describe the community of organisms which is stimulated to "bloom" proportions by addition of sugars and simple organic compounds to natural waters and predominated by bacteria of the genus *Sphaerotilus*.

These masses vary in appearance from a dirty gray to brown (due to occluded silt and diatoms) becoming black on decomposition; at times they may even be green due to associated algae. They are usually observed as a fur-like coating on rocks, twigs and debris near the surface of the water. Some investigators (Velz and Gannon, 1963) believe there are two growth types—the attached, likened to a trickling filter, and the dispersed or free-floating, similar to an activated sludge system. It is the belief of the author that the attached type is the healthy, viable growth while the floating material is usually non-viable and partially decomposing. It is possible, however, that under scouring velocities or when the growth becomes too massive to maintain its physical anchor, it may break away and continue to grow and even form a secondary growth zone downstream (Butcher, 1932).

These detached floating growths are a serious nuisance at times rendering fishing impossible due to fouling of nets and lines, such as witnessed by the author in the Altamaha River in Georgia. They may also settle out, forming a bottom sludge with serious demands on the oxygen resources of the overlying water thereby producing a secondary pollutional zone far removed from the source that produced them.

Velz and Gannon (1963) proposed that biological flocs may remove organic nutrients by biophysical contact from the overlying water and store this material within the mass without utilizing oxygen. This biochemically oxidizable material then later may exert an abnormally high oxygen demand on the stream. This biological extraction and accumulation phenomenon has not been confirmed in pure culture studies (Kirschner, 1966) although it has not been investigated thoroughly in crude biological flocs.

Sphaerotilus predominated-flocs have been reported (Smit, 1934; Lackey and Wattie, 1940; and Pipes, 1966) to cause bulking in activated sludge systems.

The first published description of *Sphaerotilus natans* was that of Kützing (1833) who collected a floc from a still bay of the Elbe near Madgeburg and described it as a new alga.

Eidam (1876) observed biological slime covering a weir in Breslau which he attributed to brewery wastes discharged upstream and identified it as *Sphaerotilus natans*. He later (1879) recognized the similarity between *Sphaerotilus* and *Crenothrix* but criticized the generalization of Cienkowski (1877) that *Cladothrix*, *Crenothrix*, *Leptothrix*, *Beggiatoa*, and all colorless filamentous bacteria were identical with *Sphaerotilus*.

Since then it has been shown (Pringsheim, 1949) that *Cladothrix dichotoma*, *Leptothrix ochracea*, *Chlamydothrix*, *Megalothrix*, and probably *Clonothrix* are synonyms of *Sphaerotilus*. Although it is generally accepted (Butcher, 1932; Bahr, 1953; Stokes, 1954; Hohnl, 1955; Mulder and van Veen, 1963) that *Cladothrix* and *Sphaerotilus* are different growth forms of the same organism, Razumov (1961) believed that most organisms presently classed in the genus *Sphaerotilus* belong in the genus *Cladothrix*. He further proposed that *Sphaerotilus* be retained only for organisms living in water polluted with complex nitrogenous compounds and which do not tolerate iron. It seems that Kützing's (1833) name alone has priority and should be retained for that reason.

Little attention was paid to the associated organisms until Büsgen (1894) was successful in growing *Sphaerotilus* in pure culture. The associations gained more notice after the formation of the "Saprobien-system" of Kolkwitz and Marsson (1908, 1909). The ecology of the floc was first studied by Butcher (1932) whose "sewage fungus" included in addition to *Sphaerotilus*, *Beggiatoa alba*, *Thiotrix niveae*, *Fusarium aquaeductum*, *Leptomitus lacteus*, *Mucor*, *Selenospora*, the protozoan *Carchesium* and the uncertain organism "*Zooglea ramigera*."

Liebmann (1951) observed the same associations within the biological floc and asserted that he could determine the age of the floc by the relative abundance of the ciliates *Colpidium colpoda* and *Glaucoma pyriformis* (= *Tetrahymena pyriformis*). Naumann (1933a, 1933b) observed similar changes and associated them with decomposition of the floc.

Perhaps the general consensus at that time was expressed by Harrison and Heukelekian (1958) when they stated, referring to the associated organisms in the biological floc, "There is no experimental evidence, however, that any of these accompanying organisms play a significant role in limiting or stimulating the development of the community."

Recently, the biological floc community was studied under simulated stream conditions by Zimmerman (1961) who employed three channels ca 243 feet long, 7.9 inches wide, and 5.9 inches deep to study the effects of velocity on biocoenosis. Each channel was constructed with a different gradient to provide velocities of ca 2.6 fps, 0.65 fps, and 0.16 fps. Sewage was fed to provide three pollution loadings, *viz*—5.5 to 6.5 ppm BOD_5, 3.3 to 3.5 ppm BOD_5, and 1.6 to 1.8 ppm BOD_5. He reported best *Sphaerotilus* growth at 2.6 fps with a BOD_5 of 5.5 to 6.5 ppm and none at a velocity of 0.16 fps at any loading nor with 1.6 to 1.8 ppm BOD_5 at any velocity. Quantitation was made of the frequency of organism generally associated with the pollution environment *viz*—*Carchesium*, *Nitzschia*, *Synedra* and *Vorticella*. *Carchesium* was most abundant at 0.65 fps and a BOD_5 of 3.3 to 3.5 ppm; *Nitzschia* abundant at 0.16 fps at a loading of 3.3 to 3.5 ppm, but not present at 2.6 fps at any loading and only at 0.65 fps and 0.16 fps at a loading of 3.3 to 3.5 ppm; *Synedra* grew best at 0.65 fps with a loading of 1.6 to 1.8 ppm showing no growth at a velocity of 0.16 fps at any loading; *Vorticella* was plentiful at a velocity of 0.16 fps and a BOD_5 of 3.3 to 3.5 ppm but did not occur at the velocity of 2.6 fps at any loading. His data indicate that current is more important than pollutional loadings in the composition of the biota in a flowing stream. Jaag and Ambühl (1963) tend to support this observation as a result of their research into the effects of current on the macro-invertebrates indigenous to flowing waters.

Wuhrmann (1964) fed sewage in concentrations of 2, 4 and 10 percent in his outdoor channels to study the effects of *Sphaerotilus* in self-purification. The channels employed in his experiments were ca 11.7 inches wide, 7.9 inches deep, 690 feet long with a slope of 0.15 percent. This provided a discharge of ca 126 gpm. His results indicated that the amount and extent of *Sphaerotilus* growth was proportional to the concentration of sewage being fed. At a sewage concentration of 4 percent, the biota of the first 495 feet consisted of *Sphaerotilus* and ciliates along with *Hormidium* and *Ulothrix* disappearing in the last 195 feet. *Diatoma vulgare* was abundant in the last 234 feet. At 10 percent *Sphaerotilus* grew throughout with *Nitzschia* and *Synedra* overgrowing in the last 360 feet. At two percent sewage, *Sphaerotilus* growth was reduced but present for 330 feet with *Diatoma vulgare* dominant in the last 195 feet. With no sewage, *Hormidium* and *Stigeoclonium* were abundant for 495 feet. *Gomphonema* and *Meridion* were present in the first 165 feet. In conclusion, he emphasized the need of simultaneous qualitative and quantitative estimates of the microorganisms present along with a measurement of self-purification of defined chemical compounds.

Harrison and Heukelekian (1958) reviewed the methods for quantitating slime growth used by various investigators pointing out the difficulties involved in sampling. These techniques involved the use of nets

(Potonie and Wundsch, 1933), glass slides (Butcher, 1946), strings (McKeown, 1962), and underwater photography (Liebmann, 1953).

The purpose of the present work was first to develop procedures which allowed reliable quantitation of growth of Sphaerotilus and associated organisms under simulated stream conditions; then to determine the effects of nutrient concentration, runoff and velocity on the amount and character of the growth, and lastly to evaluate the role of associated organisms in stimulating or inhibiting the development of a Sphaerotilus bloom. It was also hoped that any relationships between dissolved oxygen (D.O.), biochemical oxygen demand (BOD), and the biological community might be evident.

MATERIALS AND METHODS

The outdoor experimental channel (Figure 1) described elsewhere in detail (Gannon et al., 1966) was used in this investigation. In brief, the channel is composed of 4-foot repeating aluminum sections 18 inches wide, and made water-tight with a polyvinyl plastic liner. In the first series of experiments, during the summer of 1965, 645 feet were used. During the summer of 1966, 704 feet were utilized.

In the summer of 1965, sampling stations were installed at three points, No. 1 at 12 feet; No. 2 at 314 feet; and No. 3 at 625 feet (see Figure 2 for location). In the summer of 1966, three stations were maintained at the following locations: Station No. 1 at 12 feet; No. 2 at 352 feet; and No. 3 at 685 feet.

Three strands of Bear Brand Super Spun Nylon 3-ply knitting yarn (Lot No. 3401) were suspended by means of screw hooks from each station and weighted to the plastic liner by heavy hexagonal nuts to a wetted length of ca 20 cm. Figure 3 illustrates the completed installation.

After the physical and hydraulic characteristics of the channel were defined, and throughout the investigation, one string was removed from each station every 24 hours and replaced with a fresh string. In this manner it was possible to obtain (a) 24-hour strings throughout each experiment and (b) accumulation representing 24, 48, and 72 hours

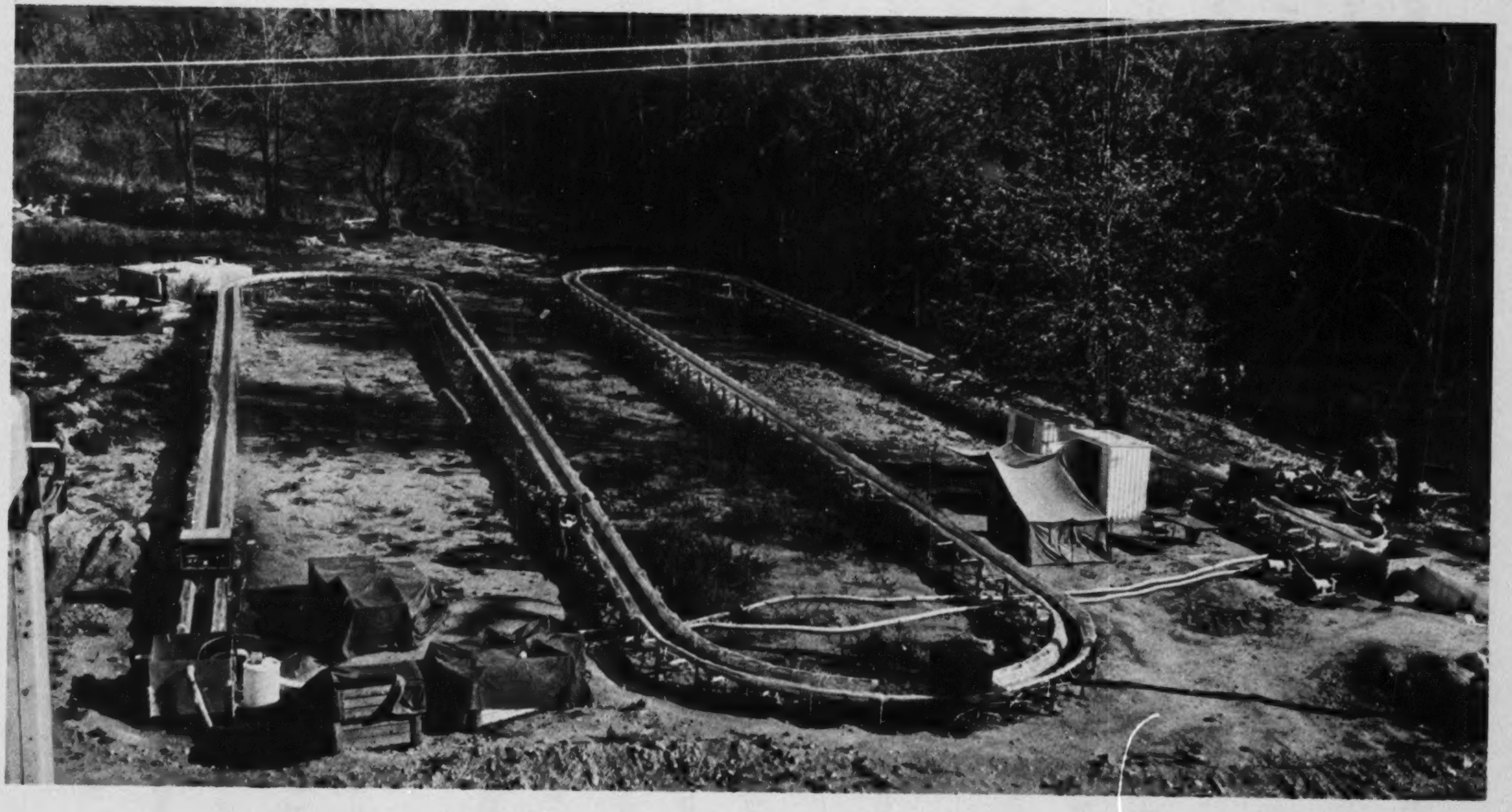


Figure 1. Aerial view of experimental channel.

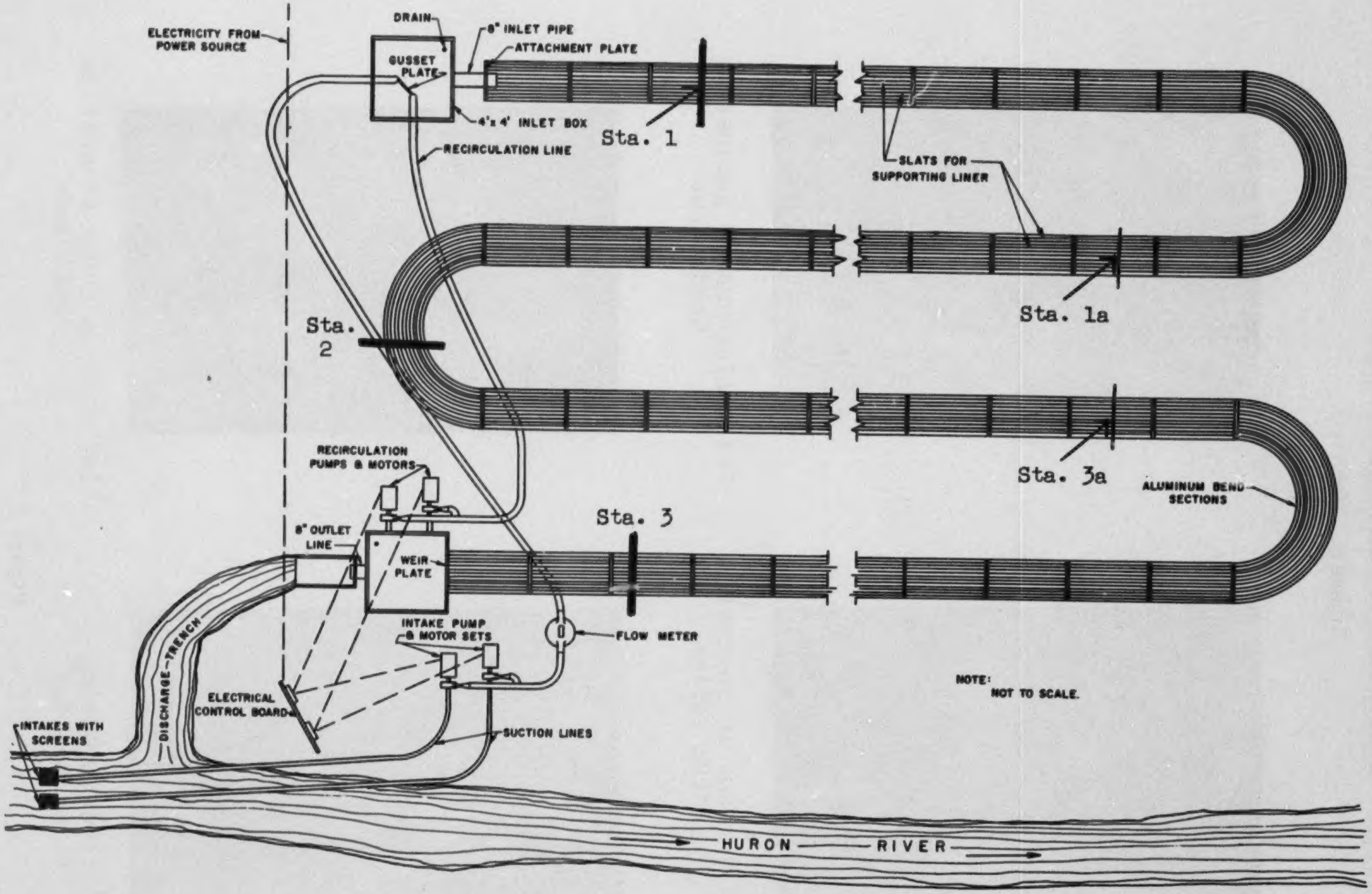
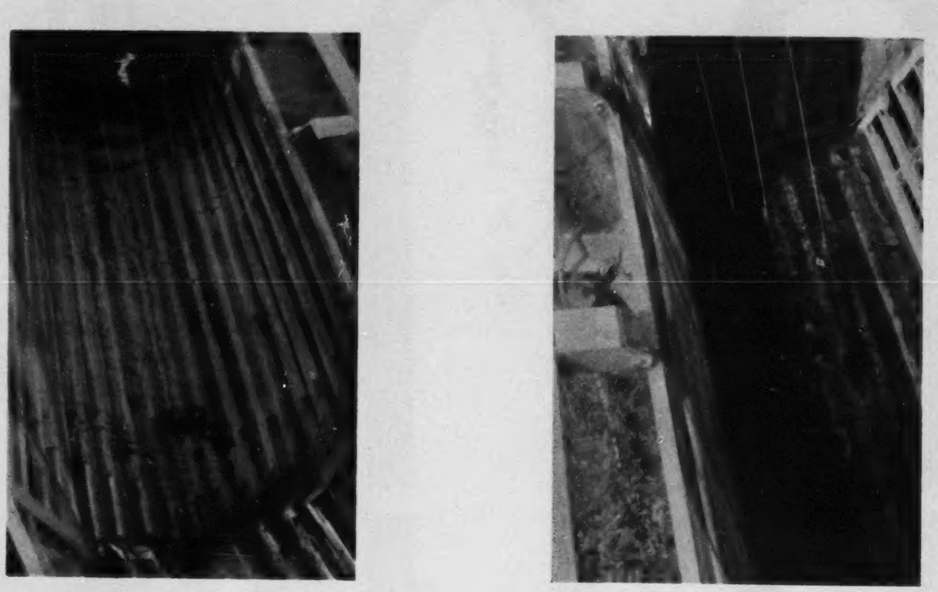

ELECTRICITY FROM POWER SOURCE
DRAIN
8" INLET PIPE
ATTACHMENT PLATE
GUSSET PLATE
4' x 4' INLET BOX
RECIRCULATION LINE
Sta. 1
SLATS FOR SUPPORTING LINER
Sta. 1a
Sta. 2
Sta. 3a
ALUMINUM BEND SECTIONS
RECIRCULATION PUMPS & MOTORS
8" OUTLET LINE
WEIR PLATE
Sta. 3
INTAKE PUMP & MOTOR SETS
FLOW METER
NOTE: NOT TO SCALE.
ELECTRICAL CONTROL BOARD
DISCHARGE TRENCH
INTAKES WITH SCREENS
SUCTION LINES
HURON RIVER

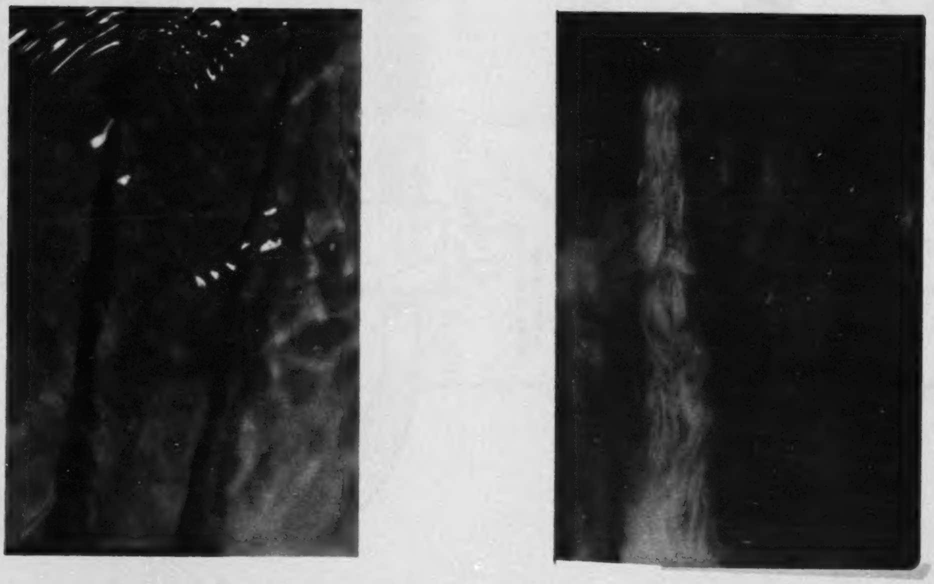
Figure 2. Location of biological sampling stations.

EXPERIMENTAL OUTDOOR CHANNEL
Biological Attachment Strings
Summer, 1965



(a) Strings at Initial Installation

(b) Strings Under Saturation Conditions



(c) Closeup During Slime Buildup

(d) Closeup Under Saturation Conditions

Figure 3

growth. However, it was thought that the 24 hour strings were more representative since older strings contained mostly debris and silt, so all three strings were harvested from each station every 24 hours during the summer of 1966. This practice also provided replicate samples for both dry weights and microscopic determinations.

At the beginning of the experiment plankton samples were taken daily from an influent sampling valve on the pump and at the overflow weir near the outlet structure. Later, it was found that the plankton apparently did not change significantly in the short time of passage through the channel, so only influent plankton was collected with occasional checks of the effluent. The procedure consisted of removing 3 to 5 liters of water and concentrating to 30 ml by means of a No. 20 plankton net. Further concentration and enumeration of organisms was performed following Lackey's (1938) drop sedimentation technique. Early in the research, samples were preserved in formalin to a final concentration of 4 percent. This was later discontinued and samples were examined within 30 minutes of collection.

From the strings, two 10 cm sections were measured and cut. One string with attached growth was resuspended in approximately 4 ml of river water and violently shaken. After discarding the string, the exact number of drops in the sample was counted. One drop was removed with a small bore pipette, placed on a clean glass slide and covered with a No. 1 cover slip. Thus by counting the organisms in one drop and multiplying by the number of drops in the concentrate, it was possible to determine the number of organisms per 10 cm of string.

The remaining 10 cm sections of string were placed into weighed aluminum pans, dried overnight in a drying oven at 90°C and the dry weight determined. Then by subtracting out the known dry weight of unused 10 cm sections of strings (which was remarkably uniform) the dry weight of the growth per se was obtained.

Standard 5-day BOD determinations were made twice daily at each station during every experiment in accordance with Standard Methods (1965).

Dissolved oxygen readings were made twice daily, using a Precision Galvanic cell oxygen analyzer (Precision Scientific Company) calibrated by the standard Winkler method. These determinations were made at 20-foot intervals so that a profile could be constructed.

Temperature was also measured twice daily at each station using a standard mercury thermometer.

Ammonia, nitrate, nitrite and total nitrogen, along with orthophosphate determinations, were made daily on the influent and effluent channel water in accordance with Standard Methods (1965).

Velocities were varied by changing the height of the overflow weir, and velocity determinations made using a current meter at twenty-foot intervals after each alteration in weir height. Times of passage were measured using a Turner Fluorometer and Rhodamin B dye.

Table 1 summarizes the characteristics of each station.

Several sets of hydraulic characteristics were used in this research. In the first series of experiments (A-F), flow was maintained at 100 gpm free flow on a straight pass through basis giving essentially a uniform velocity of one foot/second. In the second series, flow was at 50 gpm with the overflow weir varied to secure velocities from 0.8 fps to 0.1 fps at the stations.

The first series consisted of seven experiments with sucrose concentration as the only variable (except temperature, of course). These concentrations were 0, 1, 5, 10, 20, and 40 mg/liter and 5 mg/liter sucrose in the form of crude molasses.

In the second series, 5 mg/liter sucrose was used and the hydraulic characteristics changed as outlined above.

A uniform procedure of operation was adopted which consisted of cleaning the channel thoroughly, admitting river water only at the desired flow for two days, then feeding sucrose to give the desired concentration for four and one-half days.

TABLE 1

CHARACTERISTICS OF BIOLOGICAL SAMPLING STATIONS

Expt.	Weir height	Station number	Distance in feet	*Depth in inches	*Velocity in fps	*Time of passage
A-F	0.0'	1	11.5	5-1/2	1.0	0'12"
A-F	0.0'	2	314.3	5-1/2	1.0	5'15"
A-F	0.0'	3	624.5	5-1/2	1.0	10'30"
G, H	0.6'	1	11.5	3-15/16	0.71	0'18"
G, H	0.6'	1a	205.3	4-7/16	0.56	5'55"
G, H	0.6'	2	314.3	4-5/8	0.68	9'30"
G, H	0.6'	3	624.5	7-1/4	0.25	30'00"
I	0.0'	1	12	5-1/4	1.0	0'12"
I	0.0'	2	348	6	1.0	5'08"
I	0.0'	3	674	5-1/2	1.0	11'14"
J	0.6'	1	12	5-1/8	0.47	0'25"
J	0.6'	2	348	5	0.29	15'12"
J	0.6'	3	674	7-5/8	0.09	40'06"
K	0.4'	1	12	3-1/2	0.50	0'24"
K	0.4'	2	348	4	0.58	11'11"
K	0.4'	3	674	5-7/8	0.39	22'18"
L	0.2'	1	12	3-5/8	0.76	0'16"
L	0.2'	2	348	3-7/8	0.72	7'50"
L	0.2'	3	674	4-1/4	0.67	16'03"
M	0.0'	1	12	5-1/4	1.0	0'12"
M	0.0'	2	348	6	1.0	5'08"
M	0.0'	3	674	5-1/2	1.0	11'14"
N	0.0'	1	12	7-1/4	0.99	0'12"
N	0.0'	2	348	8	1.04	5'30"
N	0.0'	3	674	6-1/4	1.48	10'00"

*** During growth there was an increase in depth of from 10% - 50% while velocity was decreased up to 20% .**

Results of the Summer, 1965 effort have been reported in the June 1, 1962 - June 1, 1966 project progress report and will not be repeated here. This includes Tables 2-18 covering experiments A-H.

Summer 1966

The major variable in the experiments conducted during the summer of 1965 was nutrient concentration, so during the summer of 1966 the main focus was on the effects of velocity. Experiment I was a repetition of Experiment A of 1965 while Experiment M was designed to duplicate Experiment D of 1965, using molasses rather than beet sugar. Velocities were varied in the remaining experiments either by manipulating the overflow weir, or regulating the flow, or both. Nutrient concentration was held essentially constant at 5 mg/liter, except in M as mentioned above.

Plankton:

In July (Experiments I-L) the dominant plankters were *Navicula* sp., *Euglena fusca*, *Stephanodiscus*, *Melosira granulata*, and *M. varians*. *Difflugia*, *Desmidium* and *Ceratium* were also frequent members of the planktonic community. Temperatures were in the 26-28°C range.

During August (Experiments M and N) *Melosira granulata*, *Keratella* sp., daphnids and cyclopoid nauplii and copepodid stages were the most frequently observed plankton, with *Euglena*, *Ceratium*, and *Pediastrum* normally present. Water temperature ranged from 23-26°C.

Complete results of plankton determinations are presented in Table 19.

TABLE 19

HURON RIVER PLANKTON

Organisms	July				August	
	4-9	11-15	18-22	25-29	1-5	8-12
Ankistrodesmus sp.	7	x				
Asterionella formosa	6	x				
Ceratium hirundinella		10	3	10	5	5
Chlamydomonas sp.	4	x				
Chlorella sp.	x					
Closterium sp.	x					
Cocconeis sp.	x					
Cosmarium sp.	x					
Cyclops sp.	3	8	9	7	3	1
Cyclotella spp.	x					
Cymbella sp.	x					
Daphnia sp.	8	9	10	x	x	2
Desmidium sp.				3		
Diatoma vulgare	x				x	
Difflugia sp.	x	3	6	2	x	9
Dinobryon sertularia				5	6	8
Euglena fusca	x			1	9	
Euglena pisciformis				x	x	x
Euglena viridis				x	4	6
Gymnodinium sp.				x		
Keratella sp.	10	6	7	4	2	3
Mallonomas sp.						x
Melosira granulata	5	2	1	8	1	4
Melosira varians		x	2	9	x	
Microcystis aeruginosa			x	x	x	x
Navicula spp.	1	x	x	x	x	x
Nitzschia sp.				x	x	x
Oocystis sp.					x	x
Oscillatoria sp.				x	x	x
Pediastrum simplex		4	4	x	7	7
Peranema sp.		5	x			
Phacotus lenticularis				6		x
Phacus pleuronectes	9	x	x		10	x
Rotifera		x	8	x	8	
Scenedesmus sp.				x	x	x
Sphaerocystis sp.		7	x		x	
Spirogyra sp.					x	
Staurastrum sp.				x	x	10
Stephanodiscus sp.	2	1	5	x	x	
Surirella sp.	x					
Synedra acus				x	x	
Trachelomonas sp.				x		
Vorticella sp.			x			

Attached Organisms:

EXPERIMENT I (5 mg/1 sucrose as molasses)

This experiment was a repetition of experiment A of 1965.

Before nutrient feed began, the attached population at station number 1 was largely Melosira granulata (27%) and M. varians (18%) with Navicula, Phormidium and Spirogyra at 5% each. Also present were Cymbella, Nitzschia, Oscillatoria, Scenedesmus and Stigeoclonium. The chief macroinvertebrates were Tendipes sp., nematodes and hydropsychids. In all, there were 24 genera composed of 12,000 individuals per 10 cm with a total mean dry weight of 54.1 mg.

After 24 hours of feeding nutrient, there was a reduction in numbers of Cymbella; Spirogyra disappeared while Nitzschia increased to 10%; Oscillatoria increased to 8% and Stephanodiscus, Schroederia, Ulothrix, Euglena, Tetrahymena and Sphaerotilus made their appearance. The macroinvertebrates changed very little. Mean dry weight did not change significantly, increasing to 59.4 mg per 10 cm of string.

At the end of 48 hours, Sphaerotilus had increased to 75% of the total population; Bodo, Peranema and Colpidium appeared and Chlamydomonas, Cymbella, Cyclotella, Diatoma, Difflugia, and Oscillatoria were no longer present. The number of genera decreased to 19 while the number of individuals increased tremendously. Total mean dry weight was 173 mg per 10 cm of string.

Three days after nutrient feed began, Sphaerotilus had increased to 90% of the total population, while there was a decrease in individuals of other genera. Hydropsychid larvae were decreased while nematodes and Tendipes increased considerably. Mean dry weight was 238.2 mg.

On the fourth day of feeding, saturation population had been attained, Sphaerotilus amounting to about 98% of all organisms present. Dry weight could not be obtained with accuracy, but was in excess of 2000 mg per 10 cm of string. The only macroinvertebrates were Tendipes, Nais sp. and nematodes.

At station number 2, before feed was started the population was similar to that of station 1, except for the absence of Cymatopleura and Difflugia, the presence of Ulothrix in large numbers (5%), and increased numbers of the green algae, Spirogyra (8%), Stigeoclonium tenue (3.5%) and Oscillatoria (8%). There was slightly more Nitzschia (5%) and less Melosira. The macroinvertebrates were essentially the same as at station 1. Mean dry weight was considerably greater than at station 1, being 102.8 mg.

After 24 hours of feeding, there was an increase in Navicula of almost six-fold to 18%, and Stigeoclonium tenue to 50%, with most other forms decreasing. Sphaerotilus was present to an extent of 5%, with the macroinvertebrates essentially unchanged. Mean dry weight was 271 mg.

Forty-eight hours following introduction of nutrient, Sphaerotilus increased to 60%; there was a reduction in numbers of other forms, and Oscillatoria was no longer found. Strings and the sides and bottom of the channel were still visibly green due to the presence of Stigeoclonium. Mean dry weight was 450 mg.

On the third day of feed, Sphaerotilus attained 98% of the total population with only Stigeoclonium present in quantity (1%). The macroinvertebrates were Tendipes and a few nematodes. Gas bubbles, presumably photosynthetic oxygen, were seen within the flocs, but the algae were heavily covered with Sphaerotilus. Dry weight decreased to a mean value of 202 mg probably due to loss of attached algae. Tendipes, (the only macroinvertebrates) had increased greatly.

At the end of the fourth day of feed, saturation population had been reached, with sloughing and blackening of the attached slime. Dry weight could not be obtained due to decomposition and loss of growths. Tendipes were abundant.

At station number 3, the attached population at the time feed was started was similar to the other stations, except that Closterium, Cymbella, Cymatopleura, Cyclotella, and Pediastrum were lacking, while Ulothrix, Spirogyra and Microspora were more abundant (30%, 15% and 20%, respectively). Mean dry weight was 95.8 mg per 10 cm of string.

Twenty four hours later, Sphaerotilus appeared at 3%, Microspora increased to 30%, Stigeoclonium to 5% with no change in Spirogyra or

Ulothrix. There was a general decrease in other organisms. Accurate dry weights could not be obtained because of the mass of the green algae, but it was in excess of 1000 mg per 10 cm.

After 48 hours of feeding molasses, Sphaerotilus increased to 40%, Stigeoclonium to 40%, while Spirogyra disappeared and other organisms were generally depressed. Tendipes were the only macroinvertebrates remaining. Again the algae interferred with accurate dry weights which were in excess of 1000 mg per 10 cm of string.

Three days after feed began Sphaerotilus increased to 60%, with a decrease in Stigeoclonium tenue to 20%. Navicula increased to 10% and the other forms remained unchanged from the previous day. Mean dry weight was 1027 mg per 10 cm.

On the fourth day, Sphaerotilus (90%) and Stigeoclonium (5%) remained along with some Cosmarium, Melosira, Bodo, Tetrahymena, Colpidium and Tendipes. Dry weight was in excess of 2000 mg per 10 cm of string.

Results of attached organism determinations appear in Tables 20, 21 and 22.

EXPERIMENT J (25 gpm at 0.6 ft.)

This experiment was started with the channel at saturation population. Essentially it was a continuation of experiment I, except that the

TABLE 20

EXPERIMENT I

CHANNEL ORGANISMS AT STATION ONE

Temp. 26-28°C; Flow 100 GPM; Weir Ht. 0.0 BOD$_5$ 10-15 mg/l

Feed - Molasses 5 mg/l as sucrose

Organisms	Day				
	1	2	3	4	5
Anabaena circinalis		x			
Bodo sp.			x	x	
Chlamydomonas sp.	x	x			
Closterium sp.	x	x	x	x	
Coelastrum sp.			x		
Colpidium sp.			x	x	x
Cosmarium sp.	x	x	2.0	x	x
Cyclotella spp.	x	x			
Cymatopleura sp.	x	x		x	
Cymbella sp.	3.0	x		x	
Diatoma vulgare	x	x			
Difflugia sp.	x	x			
Euglena pisciformis			x		
Euglena sp.		x		x	x
Fragilaria crotonensis	x	x		x	
Gomphonema sp.			x		
Melosira granulata	27.0	25.0	1.5	1.0	x
Melosira varians	18.0	15.0	10.0	5.0	x
Navicula spp.	5.0	5.0	1.5	1.0	x
Nitzschia sp.	3.5	10.0	1.5	1.5	x
Oscillatoria sp.	3.5	8.0			x
Pediastrum simplex	x	x	x	x	
Peranema sp.			x	x	x
Phacus triqueter	x	x			
Phormidium sp.	5.0				
Scenedesmus sp.	1.5	1.5	x	x	
Schroederia setigera		1.5	x		
Sphaerotilus natans		x	75.0	90.0	98.0
Spirogyra sp.	5.0				
Spirulina sp.	x	x			
Stephanodiscus sp.		1.5	x	x	x
Stigeoclonium tenue	1.5	2.0			
Synedra sp.			x		
Tetrahymena pyriformis		x	x	x	x
Ulothrix sp.		x			

TABLE 21

EXPERIMENT I

CHANNEL ORGANISMS AT STATION TWO

Temp. 26-28°C; Flow 100 GPM; Weir Ht. 0.0 BOD_5 10-15 mg/l

Feed - Molasses 5 mg/l as sucrose

Organisms	Day				
	1	2	3	4	5
Bodo sp.			x	x	
Closterium sp.	x	x	x	x	
Colpidium sp.			x	x	x
Cosmarium sp.	5.0	x	1.5	x	x
Cyclotella spp.	x				
Cymbella sp.	5.0				
Diatoma vulgare	x				
Euglena fusca		x	x	x	x
Euglena pisciformis			x		
Euglena viridis	x				
Hormidium sp.	x				
Melosira granulata	22.0	10.0	1.5	x	x
Melosira varians	16.0	5.0	5.0	x	x
Microspora sp.	x			x	
Navicula spp.	3.5	18.0	5.0	x	x
Nitzschia sp.	5.0	5.0	3.5	x	x
Oscillatoria sp.	8.0	5.0			x
Pediastrum simplex	x	x	x	x	
Peranema sp.			x	x	x
Phacus triqueter	x	x			
Phormidium sp.	1.5				
Rotifera, unidentified	x	x			
Scenedesmus sp.	1.5	x	1.5	x	
Sphaerotilus natans		5.0	60.0	98.0	98.0
Spirogyra sp.	8.0	x			
Spirulina sp.	x		x		
Stigeoclonium tenue	3.5	50.0	20.0	1.0	
Tabellaria fenestrata	x				
Tetrahymena pyriformis	x	x	x	x	x
Ulothrix sp.		5.0	x	x	x
Vorticella sp.		x	x	x	

TABLE 22

EXPERIMENT I

CHANNEL ORGANISMS AT STATION THREE

Temp. 26-28°C; Flow 100 GPM; Weir Ht. 0.0 BOD_5 10-15 mg/l

Feed - Molasses 5 mg/l as sucrose

Organisms	Day				
	1	2	3	4	5
Bodo sp.			x	x	
Chlamydomonas sp.			x		
Closterium sp.		x	x	x	
Coelastrum sp.			x		
Colpidium sp.			x	x	x
Cosmarium sp.	x	x			
Diatoma vulgare	x		x		
Euglena fusca			x		
Hormidium sp.	5.0	3.0			
Melosira granulata	10.0	5.0	x	x	x
Melosira varians	5.0	2.0	5.0	5.0	x
Microspora sp.	20.0	30.0	x	x	
Navicula spp.	8.0	2.0	8.0	10.0	
Nitzschia sp.		1.5	x	x	
Oscillatoria sp.	x	x			
Pediastrum simplex		x			
Peranema sp.			x	x	
Phacus triqueter	x	x			
Phormidium sp.	x				
Pleurosigma sp.	x				
Rotifera, unidentified		x			
Scenedesmus sp.	x	x	x	x	
Schroederia setigera			x	x	
Sphaerotilus natans		3.0	40.0	60.0	90.0
Spirogyra sp.	15.0	15.0			
Spirulina sp.	x				
Stigeoclonium tenue	x	5.0	40.0	20.0	5.0
Tetrahymena pyriformis	x	x	x	x	x
Ulothrix sp.	30.0	30.0	x	x	
Vorticella sp.		x	x	x	

flow was reduced to 25 gpm and the weir raised to 0.6 ft. to give a longer time of passage (40 min.). Molasses feed was maintained at 5 mg/liter and the only change from the usual procedure was that the channel was not cleaned prior to start-up of feed. This was done in an attempt to produce a significant D.O. sag in the shortest possible time.

Unintentionally, due to mechanical failure, molasses feed was interrupted for approximately 20 hours at the beginning of the experiment. Therefore, the first day of the experiment is considered to be the time of restarting feed.

As a result of nutrient interruption, when feed was restarted, the population at station number 1 (0.47 fps) was reduced in Sphaerotilus to 50% with Stephanodiscus (20%), Nitzschia (8%), Melosira granulata (7.5%), M. varians (5%), Cosmarium (3.5%), Euglena (2%), Phacus (1.5%) and Bodo (1%). Tremendous numbers of dead Tendipes were present and the only living macroinvertebrates were Nais sp. and countless numbers of hydrophilid coleopterans resembling Hydrophilus sp. The mean dry weight of the attached growth was 116 mg per 10 cm of string.

Twenty-four hours after feed was restarted, Sphaerotilus had increased to 80%. The associated organisms decreased proportionally, except that Tendipes were alive and increased in numbers. Influent river water was exceptionally high in colloidal and settleable solids as a result of some 3-1/2 inches of rain on the afternoon previously. The influent plankton samples consisted almost entirely of silt, sludge and debris. The channel water was so murky that light penetration was restricted to the upper half-inch or so. Dry weight was in excess of 2000 mg per 10 cm of string.

After 48 hours of feeding, *Sphaerotilus* accounted for 95% of the attached population with *Oscillatoria* (3%), *Euglena* (1%), and large numbers of hydrophillids and tendipedid larvae. The chief associated bacteria were *Beggiatoa* and spirilli. Strings were too massive for accurate weights.

By the third day *Sphaerotilus* was 98% of the population, with *Tetrahymena pyriformis* (1%) next in magnitude. There were also large numbers of *Euglena* and *Oscillatoria* present. *Phacus* present in the string mass appeared highly vacuolated and being non-motile were presumed dead. *Spirillum* sp. and *Beggiatoa* in large numbers were noted in the blackened material near the center of the string mass.

The results of attached organism determinations on the fourth day were identical to those of the third day at all stations.

At station number 2 (0.29 fps), the initial population consisted of *Sphaerotilus* (40%), *Stephanodiscus* (20%), *Melosira granulata* (15%), *Navicula* (10%), with *Amoeba*, *Bodo*, *Chilomonas*, and *Cosmarium*. *Tendipes* and hydrophilids were the macroinvertebrates. Dry weight was 78.4 mg per 10 cm of string.

After 24 hours, Sphaerotilus had increased to 70% with a reduction in numbers of the other organisms. M. varians, Chlorella, Coelastrum, Coleps and Cosmarium were no longer present. The attached string population weighed in excess of 2000 mg.

On the third day of feed, Sphaerotilus had increased to 90% of the population with Stephanodiscus (5%) the only organism remaining in quantity. Strings were too massive to remove.

Four days after feed was restarted, Sphaerotilus amounted to 98% of the population, followed in dominance by Tetrahymena (1%). Euglena and Oscillatoria were observed in quantity along with Beggiatoa and Spirillum. Nais, Tendipes and the hydrophilids were the only macroinvertebrates. Dry weights were not obtained due to the magnitude of the growths and loose attachment to the string because of decomposition in the interior of the mass.

At station number 3 (0.09fps), the initial population was Sphaerotilus (40%), Stephanodiscus (30%), Amoeba (8%) and Melosira granulata (3%) along with Bodo, Pediastrum, and Pandorina. Mean dry weight was 82 mg per 10 cm of string.

After receiving nutrient for 24 hours, Sphaerotilus increased to 60%, and Stephanodiscus dropped to 10%. Other organisms were reduced in numbers while Chlorella, Difflugia, Fragilaria, Scenedesmus and Staurastrum disappeared. Dry weight could not be obtained due to the magnitude of the growth.

On the second day, *Sphaerotilus* was 90% of the attached population; *Stephanodiscus* and *Euglena* were 2% each; *Oscillatoria* reappeared and *Sphaerocystis* was no longer present. Dry weight could not be obtained.

By the third day *Sphaerotilus* had increased to 98% of the population, which consisted of *Oscillatoria*, *Euglena*, *Bodo*, *Tetrahymena*, with the bacteria *Beggiatoa* and *Spirillum* sp. *Tendipes*, *Nais* and adult hydrophilids were the macroinvertebrates.

The populations at all stations were unchanged on the fourth and final day of the experiment. Black granular sludge with an odor of hydrogen sulfide was present throughout the channel at the conclusion of this experiment. Detailed results are presented as Tables 23, 24 and 25.

EXPERIMENT K (50 gpm at 0.4 ft.)

The initial population at station 1 (0.50 fps) before nutrient was introduced consisted of *Melosira varians*, *M. granulosa* and *Stephanodiscus* at 27%, 22%, and 18% respectively, along with *Cosmarium*, *Navicula* and *Oscillatoria* at about 5% each. Mean dry weight was 26.3 mg per 10 cm.

Twenty-four hours later *Sphaerotilus* was present but less than 1% of the total population; *Cosmarium* increased to 18%; *Stephanodiscus* to 28%; *Nitzschia* to 10% and *Navicula* to 8%, while *Melosira varians* decreased to less than 1%. *Euglena fusca* appeared at 5%. *Coelastrum*,

TABLE 23

EXPERIMENT J

CHANNEL ORGANISMS AT STATION ONE

Temp. 26-28°C; Flow 25 GPM; Weir Ht. 0.6 BOD_5 12-15 mg/l

Feed - Molasses 5 mg/l as sucrose

Organisms	Day				
	1	2	3	4	5
Beggiatoa sp.			x	x	x
Bodo sp.	1.0	x	x		
Chlamydomonas sp.	x				
Chlorella sp.	x				
Closterium sp.	x	x	x		
Coelastrum sp.			x		
Cosmarium sp.	3.5	x	x		
Diatoma vulgare	x				
Euglena sp.	2.0	2.0	1.0	x	x
Hydrophilidae	x	x	x	x	x
Melosira granulata	7.5	2.0			
Melosira varians	5.0				
Nais sp.	x	x	x	x	x
Navicula sp.	x	x	x		
Nitzschia sp.	8.0	x			
Oscillatoria spp.			3.0	x	x
Pediastrum sp.			x		
Phacus pleuronectes	1.5		x	*x	
Pleurosigma sp.			x		
Scenedesmus sp.	x	x			
Schroederia setigera	x				
Sphaerotilus natans	50.0	80.0	95.0	98.0	98.0
Spirillium sp.			x	x	x
Stephanodiscus sp.	20.0	15.0	x		
Synedra sp.	x				
Tendipes sp.	x	x	x	x	x
Tetrahymena pyriformis	x	x	x	1.0	1.5

* All individuals dead

TABLE 24

EXPERIMENT J

CHANNEL ORGANISMS AT STATION TWO

Temp. 26-28°C; Flow 25 GPM; Weir Ht. 0.6 BOD_5 12-15 mg/l

Feed - Molasses 5 mg/l as sucrose

Organisms	Day				
	1	2	3	4	5
Amoeba sp.	1.0	2.0	x		
Beggiatoa sp.			x	x	x
Bodo sp.	2.0	2.0	x	x	x
Chilomonas sp.	1.0	x	x		
Chlorella sp.	x				
Coelastrum sp.	x				
Coleps sp.	x				
Cosmarium sp.	1.5				
Euglena acus			x	x	x
Hydrophilidae	x	x	x	x	x
Melosira granulata	15.0	5.0	x		
Melosira varians	x				
Nais sp.	x	x	x	x	x
Navicula spp.	10.0	5.0	x		
Nitzschia sp.	x	x	x		
Oscillatoria spp.				x	x
Pediastrum boryanium	x	x	x		
Sphaerotilus natans	40.0	70.0	90.0	98.0	98.0
Spirillum sp.				x	x
Spirogyra sp.	x				
Stephanodiscus sp.	20.0	10.0	5.0	x	
Tendipes sp.	x	x	x	x	x
Tetrahymena pyriformis				1.0	1.0

TABLE 25

EXPERIMENT J

CHANNEL ORGANISMS AT STATION THREE

Temp. 26-28°C; Flow 25 GPM; Weir Ht. 0.6 BOD_5 12-15 mg/l

Feed - Molasses 5 mg/l as sucrose

Organisms	Day				
	1	2	3	4	5
Amoeba sp.	8.0	x	x		
Beggiatoa sp.			x	x	x
Bodo sp.	2.0	x	x	x	x
Chlorella sp.	x				
Closterium sp.	x	x	x		
Cosmarium sp.	x	x	x		
Difflugia sp.	x				
Euglena oxyuris			2.0	x	x
Fragillaria sp.	x				
Hydrophilidae	x	x	x	x	x
Melosira granulata	3.0	x	x		
Nais sp.				x	x
Navicula spp.	x	x	x		
Oscillatoria spp.			x	x	x
Pandorina sp.	1.0	x	x		
Pediastrum boryanum	1.5				
Peranema sp.	x	x	x		
Phacus pleuronectes				*x	
Pyrobotrys sp.	1.0				
Scenedesmus sp.	x				
Sphaerocystis sp.	x	x			
Sphaerotilus natans	40.0	60.0	90.0	98.0	98+
Staurastrum sp.	x				
Stephanodiscus sp.	30.0	10.0	2.0		
Tendipes sp.	x	x	x	x	x
Tetrahymena pyriformis				x	x

*** All individuals dead**

Tetrahymena and *Colpidium* made their appearance. Total dry weight was 52.2 mg per 10 cm of string.

After 48 hours of feed, *Sphaerotilus* had increased to 60% of the string population, with *Stephanodiscus* at 20% and *Cosmarium* at 10%. Next in dominance were *Melosira granulata* (3%), *Euglena fusca* (2%) and *Melosira varians* (1%). A small number of *Euplotes eurystomas* were present within the floc. Midge cases were noted attached to all strings at all stations. Dry weight was 99 mg per 10 cm.

On the third day of feed, *Sphaerotilus* increased to 85% of the attached population followed by *Stephanodiscus* (8%), *Cosmarium* (5%), and *Phacus* at 3%. *Closterium* appeared in small numbers, while amoebas were no longer found. Mean dry weight was 168.8 mg per 10 cm of string.

At the end of four days, *Sphaerotilus* amounted to 98% of the population with only *Stephanodiscus* (1%) at one percent or more of the total. The other organisms remained, but were reduced in numbers. Mean dry weight was 175.7 mg per 10 cm.

At station number 2 (0.58 fps) before feed was started, the attached organisms were *Melosira varians* (30%), *M. granulata* (20%) and *Stephanodiscus* (15%). *Cosmarium*, *Nitzschia* and *Oscillatoria* were present at 5% each. Organisms occuring here and not present at station 1 were *Aphanizomenon*, *Chlamydomonas*, *Didinium*, *Euglena fusca*, Fragillaria, Schroederia, Tetrahymena, Pediastrum, Peridinium and Rivularia. Dry weight of the attached population was 31.7 mg.

After nutrient was added for 24 hours, Sphaerotilus appeared in quantity (5%), Colpidium increased to 15%, Stephanodiscus to 30% and Nitzschia to 10% while the other organisms showed little change. Dry weight was 65.8 mg per 10 cm.

At the end of 48 hours of feed, Sphaerotilus had reached bloom proportions (70%) with Stephanodiscus and Melosira varians at 10% each. Cosmarium (5%) and Oscillatoria (2%) were still present in numbers and Ulothrix appeared at 2%. The number of genera had decreased from 28 before feed began to 10. Dry weight was 185 mg per 10 cm.

Three days after nutrient feed was started Sphaerotilus dominated the population to the extent of 90%, followed by Stephanodiscus (5%), Cosmarium (3%) and Oscillatoria (1%). Mean dry weight was 161 mg.

On the fourth day of feed, Sphaerotilus reached saturation population, amounting to 98% of the attached population. The population was essentially the same as the third day except for a reduction in numbers. The attached population had a mean dry weight of 120.4 mg.

At station number 3 (0.39 fps) before adding nutrient the string population was Melosira varians and M. granulata at 25% and 20% respectively, along with Stephanodiscus (15%), Cosmarium (6%), *Oscillatoria* (5%) and *Schroederia* (2%). Dry weight was 37.9 mg per 10 cm of string.

After 24 hours of receiving nutrient, strings showed *Sphaerotilus* at 2% of the population, *Stephanodiscus* increased to 30%, *Cosmarium* to 20%, *Nitzschia* to 5%. Other organisms generally decreased. Dry weight of 10 cm of string was 77.9 mg. One hydrophillid beetle was seen and some midge eggs appeared on the strings.

At the end of 48 hours *Sphaerotilus* accounted for 70% of the population on the strings with *Stephanodiscus* second in dominance at 10%. Still present in large numbers were *Cosmarium* (8%), *Nitzschia* (5%), *Navicula* (3%) and *Vorticella* appeared at 3%. Again large quantities of midge eggs were present with one scavenger beetle noted. Mean dry weight was 150 mg per 10 cm of string.

Three days after nutrient feed began, *Sphaerotilus* reached 90% of the total attached organisms at this station. Only *Stephanodiscus* (5%), *Cosmarium* (3%) and *Oscillatoria* (1%) remained in quantity. Neither *Melosira granulata* nor *M. varians* were observed at this time. Other than their disappearance, along with that of *Ulothrix*, there was merely a reduction in numbers from that observed on the second day. Mean dry weight was 56 mg per 10 cm of string.

On the fourth and final day of nutrient feed, *Sphaerotilus* was 98% of all the attached organisms. Although the organisms present were the

TABLE 26

EXPERIMENT K

CHANNEL ORGANISMS AT STATION ONE

Temp. 26-27°C; Flow 50 GPM; Weir Ht. 0.4 BOD_5 12-21 mg/l

Feed - Molasses 5 mg/l as sucrose

Organisms	Day				
	1	2	3	4	5
Amoeba sp.	x	x	x		
Ankistrodesmus sp.	x				
Aphanizomenon sp.		x			
Chlorella sp.	x				
Closterium sp.				x	x
Cocconeis sp.	x	x	x		
Coelastrum sp.		x	x		
Colpidium colpoda		x			
Cosmarium sp.	6.0	18.0	10.0	5.0	x
Cryptomonas ovata	x	x	x		
Cymbella sp.	x	x	x	x	x
Desmidium sp.	x	x	x		
Diatoma vulgare	x	x	x		
Difflugia sp.				x	x
Dinobryon sertularia	x				
Euglena fusca		5.0	2.0	x	x
Euplotes sp.			x		
Gomphonema sp.	x				
Melosira granulata	27.0	20.0	1.0	x	x
Melosira varians	22.0	x	3.0	x	x
Microcystis aeruginosa		x			
Navicula spp.	5.0	8.0	x	x	x
Nitzschia sp.	x	10.0	x	x	x
Oscillatoria sp.	5.0	3.0	x	x	x
Pediastrum simplex		x			
Phacotus lenticularis	x	x		x	x
Phacus pleuronectes				3.0	x
Pleurosigma sp.	x				
Scenedesmus sp.	x	x	x	x	x
Schroederia setigera	x	x	x		
Sphaerotilus natans		x	60.0	85.0	98.0
Staurastrum sp.	x				
Stephanodiscus sp.	18.0	28.0	20.0	8.0	1.0
Synedra acus	x				
Tetrahymena pyriformis		x			
Ulothrix zonata		2.0			

TABLE 27

EXPERIMENT K

CHANNEL ORGANISMS AT STATION TWO

Temp. 26-27°C; Flow 50 GPM; Weir Ht. 0.4 BOD_5 12-21 mg/l

Feed - Molasses 5 mg/l as sucrose

Organisms	Day				
	1	2	3	4	5
Ankistrodesmus sp.	x				
Aphanizomenon sp.	x				
Asterionella formosa		x			
Chlamydomonas sp.	x				
Chlorella sp.	x				
Colpidium colpoda		x			
Cosmarium sp.	5.0	15.0	5.0	3.0	x
Cymbella sp.	x	x	x	x	x
Diatoma vulgare	x				
Didinium sp.	x				
Dinobryon sertularia	x				
Euglena fusca	x	x	x	x	x
Fragilaria crotonensis	x	x	x		
Gomphonema sp.	x				
Melosira granulata	20.0	18.0	x		
Melosira varians	30.0	20.0	10.0		
Microspora sp.		x			
Navicula spp.	x	x	x	x	x
Nitzschia sp.	5.0	10.0	x	x	x
Oscillatoria sp.	5.0	3.0	2.0	1.0	x
Pediastrum sp.	x				
Peranema sp.	x				
Peridinium sp.	x				
Phacotus lenticularis	x	x			
Phacus pleuronectes	x	x		x	x
Rivularia sp.	x				
Scenedesmus sp.	x				
Schroederia setigera	x	x			
Sphaerotilus natans		5.0	70.0	90.0	98.0
Staurastrum sp.	x				
Stephanodiscus sp.	15.0	30.0	10.0	5.0	x
Tetrahymena pyriformis	x				
Ulothrix zonata			2.0		

TABLE 28

EXPERIMENT K

CHANNEL ORGANISMS AT STATION THREE

Temp. 26-27°C; Flow 50 GPM; Weir Ht. 0.4 BOD_5 12-21 mg/l

Feed - Molasses 5 mg/l as sucrose

Organisms	Day				
	1	2	3	4	5
Amoeba sp.	x	x	x		
Aphanizomenon sp.	x				
Chlamydomonas sp.	x	x			
Cocconeis sp.	x				
Colpidium colpoda		x	x		
Cosmarium sp.	6.0	20.0	8.0	3.0	x
Cyclotella spp.	x			x	x
Cymbella sp.		x	x		
Diatoma vulgare	x		x	x	x
Dinobryon sertularia	x	x			
Euglena pisciformis	x	x	x	1.0	x
Fragilaria crotonensis		x			
Gomphosphaeria aponina		x			
Mallomonas sp.	x				
Melosira granulata	20.0	15.0	x	x	x
Melosira varians	25.0	20.0			
Merismopedia sp.	x				
Navicula spp.	x	x	3.0	x	x
Nitzschia sp.	x	5.0	5.0	x	x
Oocystis sp.	x				
Oscillatoria spp.	5.0	x	x	x	x
Pediastrum boryanium	x	x			
Phacotus lenticularis	x	x	x		
Phacus pleuronectes	x	x	x	x	x
Scenedesmus sp.	x	x	x	x	
Schroederia setigera	2.0	x			
Sphaerotilus natans		2.0	70.0	90.0	98.0
Staurastrum sp.		x	x		
Stephanodiscus sp.	15.0	30.0	10.0	5.0	1.0
Synedra acus	x			x	x
Tabellaria fenestrata			x		
Vorticella sp.			3.0		

same as the third day, none amounted to 1% of the population. Mean dry weight was 150.9 mg per 10 cm.

Complete results of this experiment are presented in Tables 26, 27 and 28.

EXPERIMENT L (50 gpm at 0.2 ft)

The population at station number 1 (0.77 fps) before feed was started consisted of *Melosira varians* (30%), *M. granulata* (20%), *Cosmarium* (25%), with *Euglena fusca* and *Stephanodiscus* at 10% each. Mean dry weight was 42.5 mg.

Twenty-four hours later, *Sphaerotilus* was evident at this station, reaching 10% of the string population. *Vorticella*, *Schroederia* and the macroinvertebrates—*Tendipes*, *Macronemum* and other unidentified hydropsychids were present. Mean dry weight was 70.6 mg per 10 cm.

Forty-eight hours after feed was started *Sphaerotilus* was grossly evident at all stations and throughout the channel. The attached population was dominated by *Sphaerotilus* (90%), followed by *Melosira varians* (5%), *Cosmarium* (3%), with *Melosira granulata* and *Stephanodiscus* at 1% each. Mean dry weight was 136.7 mg.

After 72 hours *Sphaerotilus* was at saturation population, amounting to 98% of the total population. No other organisms were present in quantity, but an occasional *Cosmarium*, *Melosira*, *Nitzschia*, *Oscillatoria* and *Stephanodiscus* was observed. Dry weights had a mean value of 232.9 mg per 10 cm.

On the fourth day of feed, *Sphaerotilus* was greater than 98% of the total population. *Melosira varians* and *M. granulata*, *Stephanodiscus*, *Phacus*, *Phacotus*, *Euglena* and *Cosmarium* were present, but reduced in numbers. The interior of the string was blackened, granular and anaerobic. Dry weight could not be obtained due to the magnitude of the mass.

The initial population at station number 2 (0.72 fps) was composed of *Melosira varians* (30%), *M. granulata* (25%), *Stephanodiscus* (20%), *Cosmarium* (15%), *Euglena fusca* (5%) and *Ulothrix* (2.5%). Mean dry weight was 41.0 mg per 10 cm of string.

After 24 hours of feed, *Sphaerotilus* appeared at 10%; *Ulothrix* increased to 5%; *Oscillatoria* increased to 3% and *Euglena* was no longer found. Mean dry weight was 78.3 mg per 10 cm.

Forty-eight hours of nutrient feed produced a bloom of *Sphaerotilus* (90%), with only *Melosira varians* (5%), *Cosmarium* (2%), *Stephanodiscus* (1%) and *Ulothrix* (1%) remaining in numbers. The attached population had a dry weight of 194.0 mg per 10 cm of string.

After 3 days of receiving nutrient *Sphaerotilus* was 98% of the attached population. No other organism was present at 1% of the total. Mean dry weight was 194.9 mg.

On the fourth day Sphaerotilus was dominant to the extent of 99% with the organisms present on the third day still persisting but in reduced relative numbers. Growths were too massive to permit accurate weights.

Before feed was started at station number 3 (0.66 fps) the attached organisms were Melosira varians (30%), M. granulata (20%), Cosmarium (25%), Stephanodiscus (15%) and Ulothrix (3%). Mean dry weight of 10 cm of string was 66.5 mg.

Twenty-four hours after feed began, Sphaerotilus appeared at 15% of the total string population. Associated organisms not present before feed was started were Phacotus, Phacus, Pleurosigma, and rotifers. Tendipes were present in considerable numbers. Mean dry weight was 111.3 mg.

After being fed 48 hours, the population of this station was: Sphaerotilus (90%), Cosmarium and Stephanodiscus (2% each), Melosira varians (4%) and Ulothrix zonata (1%). 10 cm of string had a mean dry weight of 239.6 mg.

Three days after feed was started, Sphaerotilus increased to 98%, with no other forms amounting to 1% or more. Tendipes were abundant. Mean dry weight was 258.1 mg per 10 cm.

On the fourth day of feed, Sphaerotilus was 99% of the population at this station, with little change in the associated organisms from the previous day. Accurate dry weights could not be obtained.

Detailed results of Experiment L appear in Tables 29, 30 and 31.

EXPERIMENT M (10 mg/liter)

This experiment was a repetition of Experiment D of 1965, using molasses at 10 mg/liter and 100 gpm free flow.

Before nutrient was added, the populations at stations 1, 2 and 3 were practically identical. They consisted of Stephanodiscus (26%), Cosmarium (21%), Melosira granulata (18%), Nitzschia (10%), Navicula (8%), Melosira varians (6%), with the euglenoids—Euglena pisciformis (3%) and E. fusca (2%), and Phacotus lenticularis (1%). Dry weight was 40.9, 39.9 and 43.8 mg respectively at the stations.

Twenty-four hours later, the population varied less than 2% between stations, so the organisms present at station 2 were considered typical of the entire channel, as in previous free-flow experiments. Sphaerotilus appeared to the extent of 5%; Vorticella appeared at 1% of the population; Melosira varians increased to 25% while M. granulata decreased to 5%; Cymbella increased to 4% and Cosmarium to 22%. Mean dry weight was: station 1, 128.6 mg; station 2, 121.1 mg; and station 3, 160.2 mg.

On the second day Sphaerotilus increased to 80% of the total string population, with a decrease in frequency of the other forms. Mean dry weights at the three stations were respectively, 132.2 mg, 105.0 mg and 77.9 mg.

TABLE 29

EXPERIMENT L

CHANNEL ORGANISMS AT STATION ONE

Temp. 27-28°C; Flow 50 GPM; Weir Ht. 0.2 BOD_5 12-18 mg/l

Feed - Molasses 5 mg/l as sucrose

Organisms	Day				
	1	2	3	4	5
Cocconeis sp.	x				
Cosmarium sp.	25.0	25.0	3.0	x	x
Cymbella sp.	x	x			
Desmidium sp.		x			
Difflugia sp.		x			
Euglena fusca	10.0	x	x	x	x
Gomphonema sp.	x				
Hormidium sp.	x				
Hydropsychidae		x			
Macronema sp.		x			
Mallonomas sp.	x				
Melosira granulata	20.0	10.0	1.0	x	
Melosira varians	30.0	30.0	5.0	x	
Navicula spp.	x	x			
Nitzschia sp.	x	x	x	x	x
Oscillatoria sp.	x	x	x	x	x
Phacotus lenticularis	x	x	x	x	x
Phacus pleuronectes	x	x	x	x	x
Rotifera, unidentified		x			
Scenedesmus sp.	x	x	x		
Schroederia setigera		x			
Sphaerotilus natans		10.0	90.0	98.0	99.0
Stauroneis sp.	x				
Stephanodiscus sp.	10.0	20.0	1.0	x	x
Synedra acus		x			
Tendipes sp.		x	x	x	x
Vorticella sp.		x			

TABLE 30

EXPERIMENT L

CHANNEL ORGANISMS AT STATION TWO

Temp. 27-28°C; Flow 50 GPM; Weir Ht. 0.2 BOD$_5$ 12-18 mg/l

Feed - Molasses 5 mg/l as sucrose

Organisms	Day				
	1	2	3	4	5
Cosmarium sp.	15.0	15.0	2.0	x	x
Cymbella sp.	x	x	x		
Euglena fusca	5.0				
Melosira granulata	25.0	10.0	x	x	x
Melosira varians	30.0	30.0	5.0	x	x
Navicula spp.	x	x	x		
Nitzschia sp.	x	x	x	x	x
Oscillatoria sp.	x	3.0			
Phacotus lenticularis		x			
Phacus pleuronectes	x	x			
Scenedesmus sp.	x				
Sphaerotilus natans		10.0	90.0	98.0	99.0
Stephanodiscus sp.	20.0	20.0	1.0	x	x
Synedra acus		x			
Tendipes sp.		x	x	x	x
Ulothrix zonata	2.5	5.0	1.0		
Vorticella sp.	x	x	x		

TABLE 31

EXPERIMENT L

CHANNEL ORGANISMS AT STATION THREE

Temp. 27-28°C; Flow 50 GPM; Weir Ht. 0.2 BOD_5 12-18 mg/l

Feed - Molasses 5 mg/l as sucrose

Organisms	Day				
	1	2	3	4	5
Cocconeis sp.	x				
Cosmarium sp.	25.0	20.0	2.0	x	x
Cymbella sp.	x	x	x		
Desmidium sp.	x				
Diatoma vulgare	x				
Dinobryon sertularia		x			
Gomphonema sp.	x	x			
Melosira granulata	20.0	10.0	x	x	x
Melosira varians	30.0	30.0	4.0	x	x
Meridion	x	x			
Navicula spp.		x	x		
Nitzschia sp.	5.0	2.0	x	x	x
Oscillatoria sp.	x	x			
Pediastrum simplex	x	x			
Phacotus lenticularis		x			
Phacus pleuronectes		x	x		
Pleurosigma sp.		x			
Rotifera, unidentified		x			
Scenedesmus sp.	x	x			
Sphaerocystis sp.		x			
Sphaerotilus natans		15.0	90.0	98.0	99.0
Staurastrum sp.	x				
Stauroneis sp.	x				
Stephanodiscus sp.	15.0	15.0	2.0	x	x
Synedra acus	x				
Tendipes sp.		x	x	x	x
Ulothrix zonata	3.0	5.0	1.0		
Vorticella sp.		x	x	x	x

After 3 days, Sphaerotilus dominated the population to the extent of 95%. There was a large amount of floating growths indicating that saturation population had occurred. The other organisms remaining were Euglena, Nitzschia, and Stephanodiscus, all at 1%, along with countless tendipedid larvae. Mean dry weights were 108.5, 90.7, and 99.4 mg/10 cm respectively.

On the fourth day of the experiment, Sphaerotilus made up over 95% of the population with the organisms present on the third day still to be found, but considerably reduced in relative numbers. Mean dry weights were 148.8, 121, and 40.3 mg at the three stations.

Results of this experiment are presented in Table 32.

EXPERIMENT N (200 gpm)

This experiment was designed to investigate the highest velocities attainable in the system without changing the slope.

At the start of the experiment, the attached population at station number 1 (0.99 fps) was predominantly Melosira varians (50%) and M. granulata (35%) with some Oscillatoria (5%) and Euglena fusca (4%). Mean dry weight was 42.6 mg/10 cm.

After feeding molasses for 24 hours, Sphaerotilus appeared in bloom proportions (35%); Phacus appeared at 3.0%; Navicula and Nitzschia increased to 2% each; and Melosira was reduced proportionally by about 50%. Dry weight increased to a mean of 66.2 mg/10 cm.

TABLE 32

EXPERIMENT M

CHANNEL ORGANISMS AT STATION TWO

Temp. 25-27°C; Flow 100 GPM; Weir Ht. 0.0 BOD_5 20-33 mg/l

Feed - Molasses 10 mg/l as sucrose

Organisms	Day				
	1	2	3	4	5
Closterium sp.		x			
Cosmarium sp.	21.0	22.0	5.0	x	x
Cyclotella spp.	x				
Cymbella sp.	x	4.0	x	x	
Desmidium sp.	x				
Dinobryon sertularia	1.0	x			
Euglena fusca	2.0	1.0	1.0	1.0	x
Euglena pisciformis	3.0	2.0	1.0	x	x
Fragilaria crotonensis		x			
Hydropsychidae	x	x			
Melosira granulata	18.0	5.0	x	x	
Melosira varians	6.0	25.0	10.0	x	x
Mougeotia sp.	x	x	x		
Navicula spp.	8.0	5.0	x	x	x
Nitzschia sp.	10.0	8.0	2.0	1.0	x
Oocystis sp.	x				
Oscillatoria sp.	x		x		
Pediastrum simplex	x	x			
Phacotus lenticularis	1.0	x			
Phacus pleuronectes	3.0	1.0	x	x	x
Rotifera, unidentified			x	x	
Scenedesmus sp.	x	x			x
Schroederia setigera	x	x			
Sphaerotilus natans		5.0	80.0	95.0	98.0
Stephanodiscus sp.	26.0	15.0	x	1.0	x
Synedra acus	x	x			
Tendipes sp.	x	x	x	x	x
Vorticella sp.		1.0	x	x	x

On the second day of feed, Sphaerotilus increased to 98% of the population, with the associated organisms remaining, but with none at 1% of the population. Dry weight was 349.4 mg/10 cm of string.

Three days after feed was started, saturation population had occurred. Growth was so heavy throughout the channel, that the level at station 1 rose from the normal 7-1/8 in. to 11 in., or about 1/4 in. below the top of the liner. The attached population was Sphaerotilus (>99%) with a horde of Tendipes, and a few Phacus, Melosira varians, Nitzschia, Navicula, Euglena and Cosmarium. Colpidium and Tetrahymena became abundant. The strings were anaerobic in all but the outermost layer of slime, and dry weights are not considered accurate due to the mass, but they were in excess of 1500 mg/10 cm.

On the last day of the experiment, there was no change except in total mass. Dry weight could not be obtained with accuracy, but is estimated as greater than 2000 mg.

At station number 2 (1.06 fps) when the experiment began, the string population was similar to that of station 1, except for the presence of more Navicula (2%), Nitzschia (2%), and Cosmarium (10%). Mougeotia was also noted in small numbers here but not at station 1. Mean dry weight was 53.2 mg per 10 cm.

After 24 hours of feed, Sphaerotilus appeared in a massive bloom, amounting to 50% of the attached population. Mougeotia increased to 2%, and *Spirogyra*, *Oedogonium*, and *Ulothrix* appeared at 8%, 2%, and 2% respectively. Dry weight was 146.5 mg.

On the second day, *Sphaerotilus* increased to 99% and the strings were approaching saturation population. *Spirogyra*, *Oedogonium*, and *Ulothrix* were no longer present at this station. Dry weight was 700 mg/10 cm.

Three days after feed began, *Sphaerotilus* was over 99% of the attached organisms, with only *Nitzschia*, *Navicula* and *Melosira* remaining. Dry weight could not be obtained accurately, but was in excess of 1500 mg.

At the end of the experiment, the string population was unchanged from the third day except for an increase in mass. Dry weight was not obtained, but was thought to be in excess of 2000 mg/10 cm of string.

At station number 3 (1.48 fps) before nutrient was fed, the population was very similar to that of station 2. Dry weight was 43.8 mg/10 cm of string.

After being fed for 24 hours, *Sphaerotilus* was present at 55% of the population. *Oedogonium* appeared at 3%, *Ulothrix* at 5%, and *Stigeoclonium tenue* at 15%. Mean dry weight was 172.7 mg.

On the second day, *Sphaerotilus* increased to 80%, sharing dominance with *Stigeoclonium* (15%). *Ulothrix*, *Oedogonium*, *Melosira granulata* and *Nitzschia* were no longer observed in the string population.

Tendipes were abundant on the strings and sides of the liner. Mean dry weight was 1180.5 mg.

Three days after feed began, Sphaerotilus dominated the population to the extent of 99%. Stigeoclonium (1%) was the only organism remaining in quantity. Dry weight was in excess of 1500 mg/10 cm of string.

On the fourth and final day, the attached population was 99% Sphaerotilus with small amounts of Stigeoclonium, Nitzschia, Cosmarium, the ciliates—Colpidium and Tetrahymena, and of course the tendipedid larvae. Growths were too decomposed to harvest, but were estimated to exceed 2000 mg/10 cm in dry weight.

Dissolved Oxygen:

Dissolved oxygen content of influent river water was continuously measured using a galvanic cell oxygen electrode connected to a strip chart recorder. DO concentration varied from above saturation to almost zero during the summer, but generally followed a typical diurnal curve with the maximum peak at 12 noon to 3:00 p.m., and the minimum around 3:00 a.m. Figures 4-8 are plots of the influent DO for Experiments J-N.

Channel dissolved oxygen measurements were made using a Beckman Oxygen Analyser, after difficulty was experienced with the Precision instrument. Reaeration in the channel was good, producing an increase

TABLE 33

EXPERIMENT N

CHANNEL ORGANISMS AT STATION ONE

Temp. 23-26°C; Flow 200 GPM; Weir Ht. 0 BOD$_5$ 15-30 mg/l

Feed - Molasses 5 mg/l as sucrose

Organisms	Day				
	1	2	3	4	5
Anabaena sp.		x			
Closterium sp.				x	
Colpidium sp.				x	x
Cosmarium sp.	x	x	x	x	x
Cymbella sp.	x	x	x		
Difflugia sp.	x	x	x	x	
Euglena fusca	4.0	5.0	x	x	x
Melosira granulata	35.0	20.0			
Melosira varians	50.0	25.0	x	x	x
Navicula spp.	x	2.0	x	x	x
Nitzschia sp.	x	2.0	x	x	x
Oscillatoria sp.	5.0	5.0			
Pediastrum sp.		x			
Phacotus lenticularis		x			
Phacus sp.		3.0	x	x	x
Pleurosigma sp.	x	x			
Scenedesmus sp.	x	x			
Sphaerotilus natans		35.0	98.0	99+	99+
Spirogyra sp.		1.0	x		
Stauroneis sp.	x	x			
Stigeoclonium tenue		x			
Tetrahymena sp.		x	x	x	x
OTHER ORGANISMS	(ACTUAL NUMBERS PER 10 CM)				
Hydropsychidae		2			
Macronema sp.		2			
Nematoda		30			
Tendipes sp.		50	75	75	25

TABLE 34

EXPERIMENT N

CHANNEL ORGANISMS AT STATION TWO

Temp. 23-26°C; Flow 200 GPM; Weir Ht. 0 BOD$_5$ 15-30 mg/l

Feed - Molasses 5 mg/l as sucrose

Organisms	Day				
	1	2	3	4	5
Cosmarium sp.	10.0	x	x		
Cymbella sp.	x	x	x		
Diatoma vulgare		x			
Euglena fusca	2.0	x			
Euglena viridis			x		
Melosira granulata	30.0	2.0	x	x	x
Melosira varians	45.0	25.0	x	x	x
Meridion sp.		x			
Mougeotia sp.	x	2.0	x		
Navicula spp.	2.0	2.0	x	x	x
Nitzschia sp.	2.0	2.0	x	x	x
Oedogonium sp.	x	1.0			
Oocystis sp.	x				
Oscillatoria sp.	x				
Phacus pleuronectes	x			x	x
Pleurosigma sp.	x				
Scenedesmus sp.	x				
Sphaerotilus natans		50.0	99.0	99+	99+
Spirogyra sp.		8.0			
Stauroneis sp.	x	x			
Stephanodiscus sp.		x	x		
Synedra acus		1.0			
Ulothrix zonata		2.0			
OTHER ORGANISMS	(ACTUAL NUMBERS PER 10 CM)				
Nematoda			20	10	2
Tendipes sp.		10	35	50	20

TABLE 35

EXPERIMENT N

CHANNEL ORGANISMS AT STATION THREE

Temp. 23-26°C; Flow 200 GPM; Weir Ht. 0 BOD_5 15-30 mg/l

Feed - Molasses 5 mg/l as sucrose

Organisms	Day				
	1	2	3	4	5
Colpidium sp.				x	x
Cosmarium sp.	10.0	1.0	x	x	x
Cymbella sp.	x	1.0	x		
Euglena fusca		x	x		
Fragilaria crotonensis	x				
Melosira granulata	30.0	x			
Melosira varians	40.0	10.0	3.0	x	
Microcystis aeruginosa		x			
Navicula spp.	3.0	x			
Nitzschia sp.	2.0	1.0	x	x	x
Oedogonium sp.	x	3.0			
Oscillatoria sp.	5.0	x	x		
Pediastrum simplex	x				
Phacus triqueter	x			x	
Pleurosigma sp.	x	x			
Scenedesmus sp.	x				
Schroederia setigera	x				
Sphaerotilus natans		55.0	80.0	98.0	99+
Stauroneis sp.	x				
Stephanodiscus sp.		x	x		
Stigeoclonium tenue		15.0	15.0	1.0	x
Tetrahymena sp.				x	x
Ulothrix zonata		5.0			
OTHER ORGANISMS	(ACTUAL NUMBERS PER 10 CM)				
Nematoda	3	10	5	5	
Tendipes sp.	3	20	20	35	10

INFLUENT RIVER DISSOLVED OXYGEN

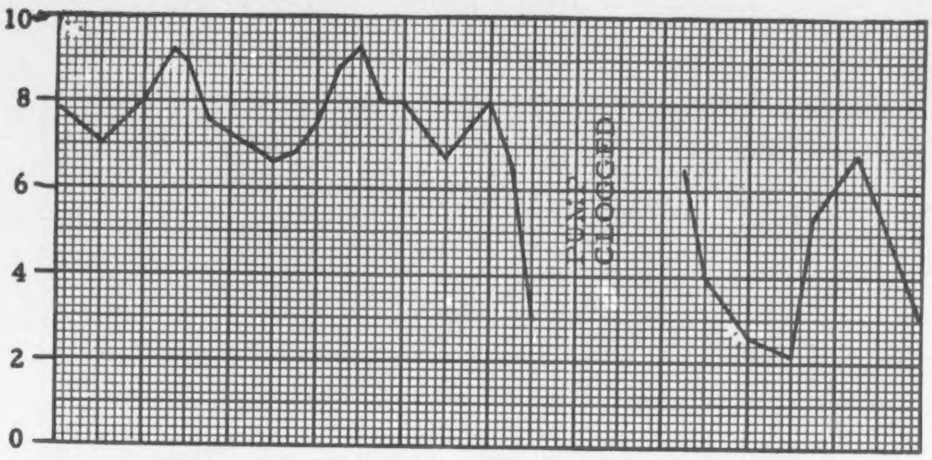


Figure 4. Experiment J

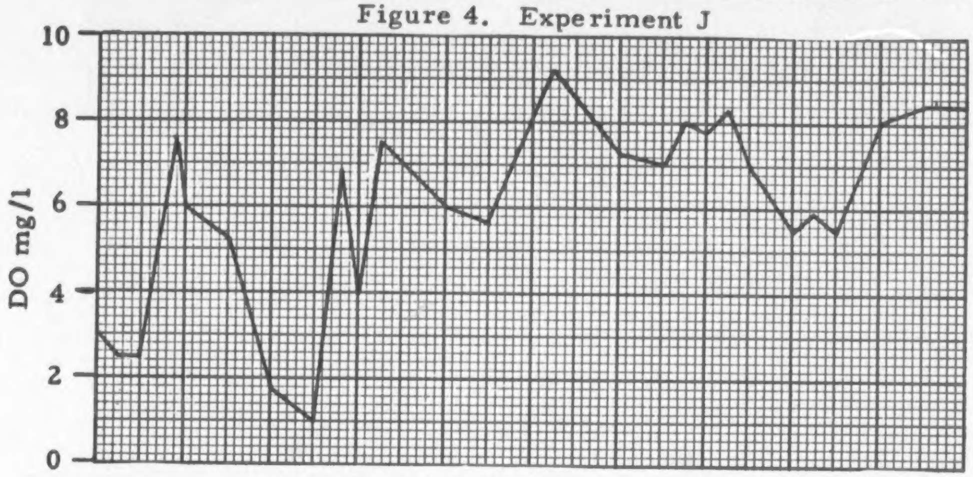


Figure 5. Experiment K

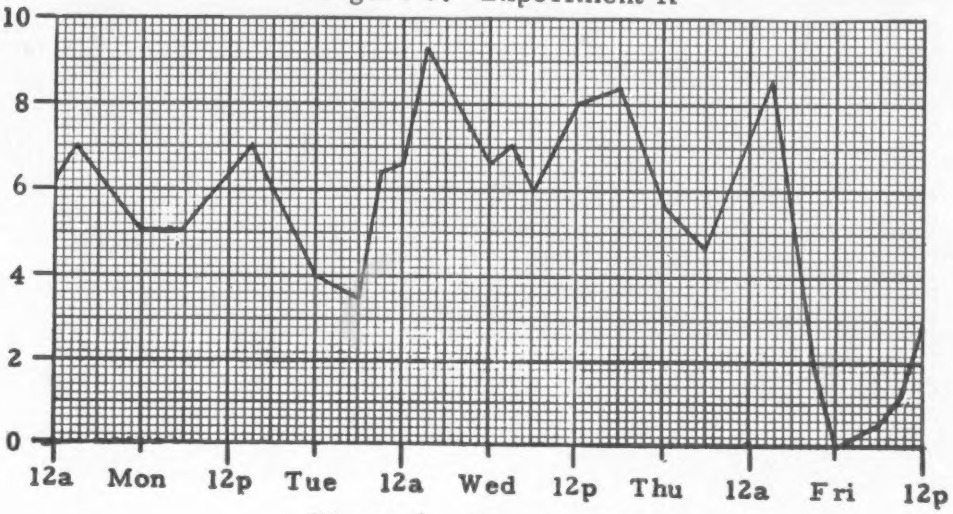


Figure 6. Experiment L

INFLUENT RIVER DISSOLVED OXYGEN

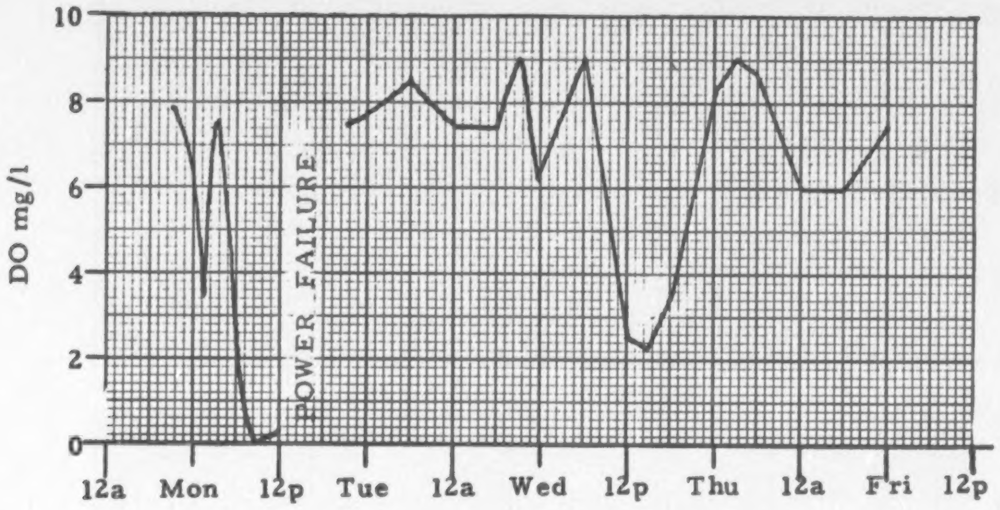


Figure 7. Experiment M

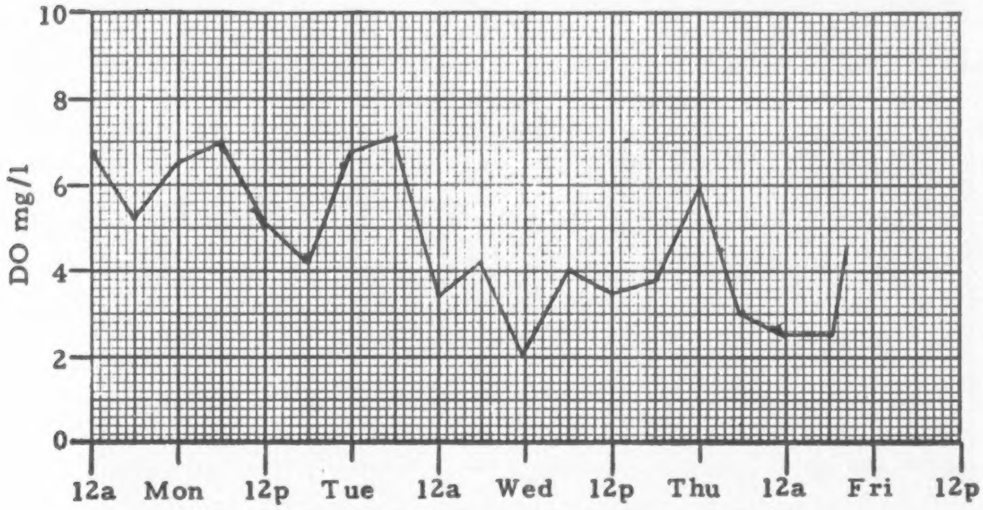


Figure 8. Experiment N

of up to 2.2 mg/liter in DO. The daytime k_2 values reported elsewhere for 1965 (Gannon, et al., 1966) for approximately 11 min. time of passage had a mean of 14.6/day, and a range from 10.0-17.2/day. Night k_2/day was 7.8 and 8.4 on two runs, indicating that almost half of the total reaeration was due to photosynthesis.

One objective of the effort in 1966 was to demonstrate a "DO sag" in the channel and Experiment J was expressly designed for this purpose. A sag did occur, developing between 180 and 220 feet, increasing in magnitude of depression to a maximum of 2.4 mg/liter on the fourth day of the experiment. In fact, DO depressions were demonstrable in all experiments except K, varying from 1.0 mg/liter up to 4.1 mg/liter. Usually the sag reached a maximum just after saturation population had occurred.

Figures 9 and 10 are comparisons of the DO profiles for the first and fourth day of feed during Experiments L and N. Sags obtained in other experiments followed this same general pattern.

Dissolved oxygen results are presented in tabular form later in this section.

Biochemical Oxygen Demand:

Considerable difficulty was experienced with the standard 5 day BOD determinations throughout this research.

The BOD_5 as determined in the laboratory, of a 5 mg/liter solution of molasses in river water was about 12 mg/liter. On this basis,

Figure 9. Channel DO Profile, Experiment L

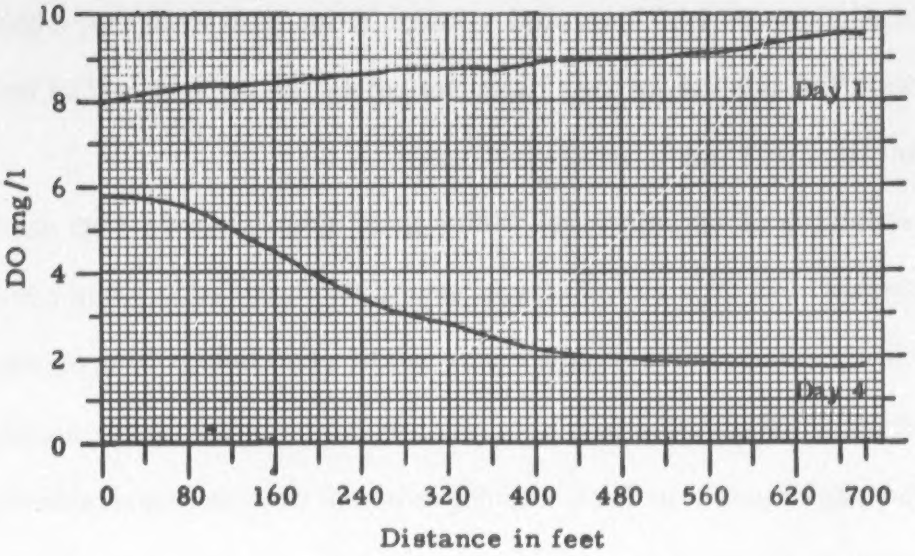
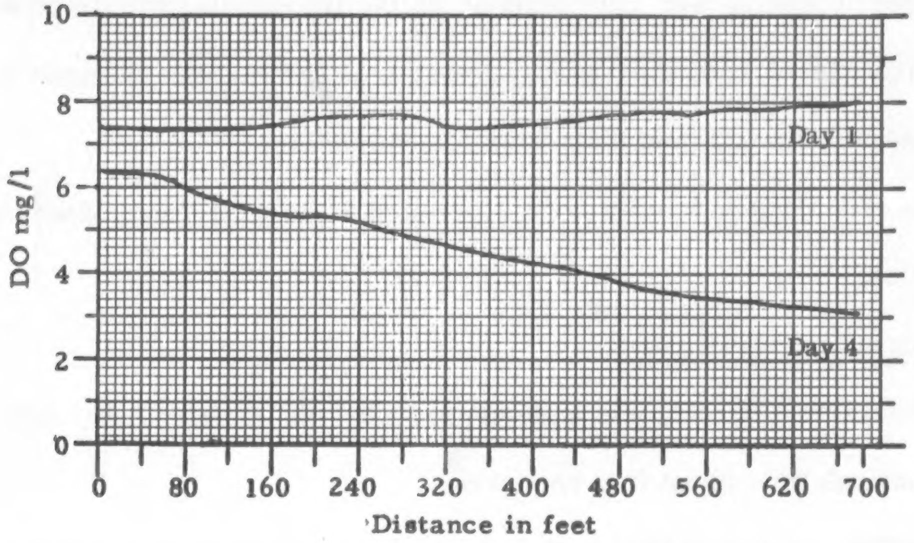


Figure 10. Channel DO Profile, Experiment N



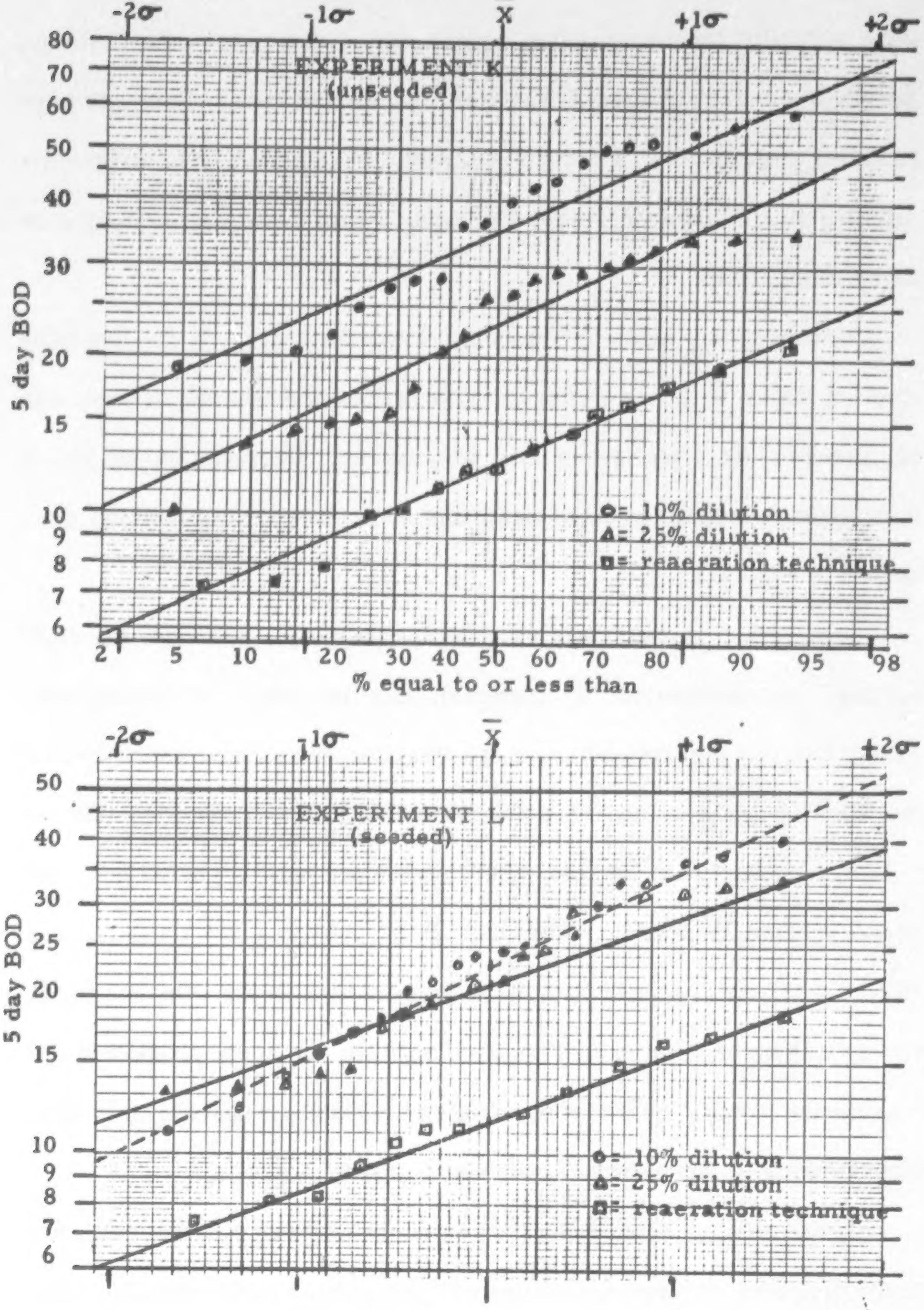
dilutions of 50% and 25% were used. In most cases, the 25% dilutions gave virtually the same 5 day oxygen depletions as the 50% dilutions, resulting in a calculated BOD twice that of the latter. This was particularly discouraging when 10% dilutions were added, with further increases in the apparent BOD. Depletion in the blank was usually less than 0.2 mg/liter.

An attempt was made to overcome this difficulty by: (1) seeding BOD dilution water with 1 ml/liter of raw settled and filtered sewage; and (2) using a modification of the Elmore jug reaeration technique. A comparison of BOD results using these methods is graphically presented in Figure 11.

In the latter method, full strength samples were split into two BOD bottles, one designated "S" (sample), and the other "R" (reservoir). Initial DO was determined using the Beckman instrument calibrated by the Winkler method, and then determined every 24 hours for 5 days in a like manner. Any sample liquid displaced was replaced from the corresponding R bottle. When "S" DO approached 2.0 mg/liter, the "R" and "S" bottles were combined, aerated by pouring back and forth, and DO again determined. This method gave the most consistent and reasonable results in the study. It also provided the added potential of furnishing daily laboratory deoxygenation rates.

Another serious source of error in the BOD determinations was due to the presence of large quantitites of Sphaerotilus in the samples after

Figure 11. Effects of Dilution and Seeding on BOD



saturation population occurred in the channel. This material was shown in preliminary laboratory studies to have a tremendous oxygen demand following death and decomposition. It also has a very high iodine demand, seriously interferring with dissolved oxygen determinations by the Winkler method. The latter difficulty was overcome by using the Beckman analyser for BOD determinations, but no solution was found for the former difficulty. There seems to be no practical nor effective filtration, flocculation, or sedimentation method for removing this growth. Since it could not be uniformly distributed in samples, it was responsible for many erratic results as well as the apparent lack of BOD reduction between the stations in some cases.

The data obtained using the reaeration technique were subjected to several forms of analysis in an attempt to arrive at a laboratory deoxygenation constant (k_1) for the channel water. All methods used gave different values, and none fit the observed rates very closely. Inspection of the data indicated little difference between stations, but considerable variation between days of the experiment. Therefore, it was decided to assign a value of 100% to the 5 day figures and calculate daily percent removal on that basis. Then a mean daily removal percentage was calculated for the three stations during three experiments (K, L, and M) which were all run at 5 mg/l. Therefore, the percentages appearing in Table 36 are actually the means of nine individual per-

TABLE 36

SUMMARY OF LABORATORY BOD REMOVAL RATES

Day of Expt.		Day of Incubation				
		1	2	3	4	5
		Percent Removed				
1	$\bar{X}$	13.6	39.1	49.4	69.1	100
	Range	10.3-18.2	25.6-44.3	44.3-59.7	64.2-77.8	
2	$\bar{X}$	16.5	43.0	64.7	86.3	100
	Range	7.6-23.9	32.0-48.2	48.8-75.9	78.2-89.2	
3	$\bar{X}$	25.3	47.8	68.3	83.1	100
	Range	16.5-31.2	34.5-60.4	50.7-99.6	70.5-92.7	
4	$\bar{X}$	24.2	50.6	66.9	88.9	100
	Range	11.0-38.0	41.9-54.2	64.2-74.4	83.8-90.3	
5	$\bar{X}$	21.9	42.3	56.0	85.7	100
	Range	14.0-27.4	33.4-51.4	40.6-65.1	80.9-91.3	

centages. These are not true rates in the sense of velocity constants but they demonstrate very well that BOD removal was considerably more rapid in the samples taken on the fourth day of the experiments, than on preceding days, and slowest on the first day samples. This seems to indicate that bottles containing a great deal of Sphaerotilus at saturation population have higher rates than those incubated in the absence of Sphaerotilus. This observation will be discussed further in a later section.

BOD reduction between the stations was greatest in most cases just before saturation population occurred, decreasing thereafter. In terms of percent removal, reduction at saturation population ranged from 5.68%-45.7%. Expressed as k_c per day where:

$$k_c = \frac{\text{log BOD at Sta. 1 - log BOD at Sta. 3}}{\text{time between Stas. in days}},$$

these values were from 3.6-31.0 per day.

Maximum BOD removal values are summarized for each experiment in Table 37.

DO—BOD Relationships:

Table 38 is a summary of the DO and BOD data for Experiments I-N. Generally, it may be seen that while maximum BOD removal generally occurred at or just prior to saturation population, decreasing afterward, DO depression usually occurred following saturation population and increased thereafter.

TABLE 37

MAXIMUM BOD REMOVAL IN CHANNEL, SUMMERS 1965-1966

Expt.	Day	Temp.	Conc. mg/l	Time of Passage	Feed*	BOD Removed	% Removed	k_c	ΔDO
A	3	26	5	10'50"	M	3.7	45.7	31.0	0
B	3	23	40	10'50"	S	14.4	28.0	18.86	+0.4
C	3	19.5	20	10'50"	S	6.1	27.8	18.80	-0.7
D	3	21.3	10	10'50"	S	3.6	31.0	21.52	+0.5
E	3	24.0	5	10'50"	S	3.8	24.0	22.98	+0.6
F	1	15.5	1	10'50"	S	2.4	33.8	23.89	+0.6
G	2	10.8	10	30'00"	S	2.4	37.0	9.34	-0.4
H	8	14.0	5	30'00"	S	3.0	41.0	10.68	+0.6
I	5	26.0	5	11'14"	M	1.5	8.35	4.75	+0.6
K	4	26.0	5	22'18"	M	5.6	35.9	12.3	+0.9
L	4	27.0	5	16'03"	M	§5.2	§39.1	§34.0	-3.3
M	4	27.0	10	11'14"	M	14.5	26.4	17.1	-2.3
N	2	26.0	5	10'00"	M	3.0	5.68	3.6	-0.7

* M = Molasses
S = Beet Sugar
§ = Reaeration method; all others by Standard Methods

TABLE 38

SUMMARY OF DO AND BOD DATA—SUMMER 1966

Expt.	Day	Time	Temp.	Station 1		Station 2		Station 3		ΔDO	ΔBOD
				DO	BOD	DO	BOD	DO	BOD		
I	1	AM	28°C	-	-	-	-	-	-	-	-
		PM		-	10.3	-	10.5	-	10.8	-	-
	2	AM	28°C	7.35	13.8	7.40	13.0	7.95	13.7	+0.6	-0.1
		PM		8.90	19.6	9.70	20.2	11.00	19.2	+2.1	-0.4
	3	AM	27°C	6.70	25.2	7.70	25.9	8.90	-	+2.2	-
		PM		7.70	26.4	8.20	26.4	8.70	26.1	+1.0	-0.3
	4	AM	26°C	8.30	24.2	7.90	23.7	7.90	23.0	-0.4	-1.2
		PM		8.80	x	8.10	x	7.80	x	-1.0	x
	5	AM	26°C	6.20	18.4	6.80	17.5	6.80	16.9	+0.6	-1.5
		PM		8.20	x	7.10	x	6.70	x	-1.5	x
J	1	AM	27°C	6.75	x	7.15	x	7.40	x	+0.65	x
		PM		7.80	x	7.45	x	7.60	x	-0.20	x
	2	AM	27°C	7.50	x	6.45	x	6.65	x	-0.85	x
		PM		8.00	x	6.20	x	6.40	x	-1.60	x
	3	AM	27°C	6.10	x	4.35	x	4.70	x	-1.40	x
		PM		6.00	x	4.40	x	4.70	x	-1.30	x
	4	AM	27°C	6.50	x	4.20	x	4.10	x	-2.40	x
		PM		6.50	x	4.70	x	4.80	x	-1.70	x
	5	AM	25°C	6.70	x	5.35	x	5.55	x	-1.15	x
K	1	AM	26°C	8.40	*21.0	8.80	*17.9	9.20	*19.0	+0.8	*-2.0
		PM		8.10	52.0	8.70	53.5	9.20	55.5	+1.1	-3.5
					*15.9		*15.4		*16.5		*-0.6
	2	AM	27°C	8.00	20.5	8.60	22.4	9.50	21.3	+1.5	-1.8
		PM		8.10	31.0	8.70	32.5	9.20	35.0	+1.1	-4.0
					*13.3		*12.1		*11.4		*-1.9
	3	AM	26°C	8.00	14.2	8.70	15.0	9.30	13.6	-1.3	-0.6
		PM		8.00	43.5	8.30	42.0	8.80	47.5	+0.8	-0.4
					*12.1		*10.3		*10.0		*-2.1
	4	AM	26°C	8.10	15.6	8.40	14.8	9.00	10.0	+0.9	-5.6
		PM		8.10	36.0	8.00	27.0	8.70	35.5	+0.6	-0.5
					* 7.9		* 7.4		* 7.3		*-0.6
	5	AM	26°C	7.90	13.4	7.70	10.2	8.40	10.4	+0.5	-3.0

TABLE 38 (continued)

Expt.	Day	Time	Temp.	Station 1		Station 2		Station 3		ΔDO	ΔBC
				DO	BOD	DO	BOD	DO	BOD		
L					*18.7		*16.4		*16.9		*-1.8
	1	AM	28°C	-	x	-	x	-	24.4	-	x
		PM		8.00	40.0	8.70	51.5	9.50	49.0	+1.5	+9.0
					*11.9		*11.7		*11.1		*-0.8
	2	AM	28°C	7.10	19.0	8.20	21.0	8.30	24.4	+1.2	+5.4
		PM		7.60	43.5	8.90	35.5	9.90	36.5	+1.3	-7.0
					*11.1		* 9.5		* 7.4		*-3.7
	3	AM	28°C	6.70	14.2	6.70	13.0	7.10	12.6	+0.4	+1.6
		PM		7.50	x	7.60	x	8.20	x	+0.7	x
					*13.3		* 8.2		* 8.1		*+5.2
	4	AM	27°C	4.20	17.2	1.20	13.0	0.90	12.4	-3.3	-4.8
		PM		5.80	38.0	2.60	23.5	1.70	22.4	-4.1	-15.6
					*15.0		*13.3		*10.7		*-4.3
	5	AM	27°C	5.60	28.5	4.10	21.5	3.50	18.1	-2.1	-10.4
M					*19.4		*19.6		*19.3		*-0.3
	1	AM	26°C	-	x	-	x	-	41.0	-	x
		PM		5.50	x	6.30	x	6.90	x	+1.4	x
					* 3.6		* 3.3		* 2.8		*+0.6
	2	AM	25°C	7.40	3.2	7.90	4.2	8.70	3.0	+1.3	+0.2
		PM		7.90	51.5	9.00	51.5	9.70	57.5	+1.8	+6.0
					*13.8		*14.1		*13.9		*+0.1
	3	AM	27°C	8.00	53.5	7.60	44.5	8.70	40.5	+0.7	+13.0
		PM		7.70	x	7.40	x	6.70	x	-1.0	x
					* 7.6		*11.8		*23.5		*+15.9
	4	AM	27°C	7.70	6.6	6.80	12.6	6.30	15.2	-1.4	+8.6
		PM		8.00	55.0	7.10	45.0	5.70	40.5	-2.3	-14.5
					*12.5		*11.4		*12.1		*-0.4
	5	AM	24°C	7.70	32.0	6.80	34.0	6.00	34.0	-1.7	+2.0
N					* 4.4		* 2.3		* 2.7		*-1.7
	1	AM	26°C	-	3.8	-	3.2	-	2.0	-	-1.8
		PM		7.40	34.5	7.40	36.5	8.00	45.0	+0.6	+10.5
					* 4.1		* 4.2		* 4.2		*+0.1
	2	AM	26°C	6.80	2.4	6.80	3.2	7.10	3.2	+0.3	+0.8
		PM		6.80	53.0	6.50	x	6.10	50.0	-0.7	-3.0
					* 5.1		* 5.5		* 4.8		*-0.3
	3	AM	25°C	6.30	4.4	5.60	4.4	5.50	3.6	-0.8	-0.8
		PM		6.40	x	4.70	34.0	3.20	38.0	-3.2	x
					* 6.6		*10.2		* 7.5		*+1.5
	4	AM	23°C	6.00	4.0	5.10	4.6	4.80	13.0	-1.2	-9.0
		PM		5.50	35.5	2.90	x	1.50	x	-4.0	x

* = Reaeration method
x = Residual DO < 2 mg/l in samples
- = No samples

Nitrogen and Phosphorus:

Influent channel water varied in total nitrogen from 0.28 to 1.68 mg/liter, with a mean value of 0.85 ± 0.32 mg/liter. Nitrate nitrogen ranged from zero to 0.11 mg/liter with a mean of 0.007 mg/liter. Nitrite nitrogen varied from zero to 0.16 mg/liter with a mean of 0.016 mg/liter.

Influent ortho-phosphate ranged from 0.001 to 0.01 mg/liter.

In most cases there was a reduction in phosphate concentration through the channel in the order of 0.003 to 0.006 mg/liter, but there was no discernable pattern to the behavior of nitrogen through the channel.

Complete results of these determinations appear in Table 39. These results indicate that there was sufficient nitrogen and phosphorus present to support a normal stream biota at all times.

TABLE 39

SUMMARY OF NITROGEN AND PHOSPHORUS DETERMINATIONS, SUMMER 1966

Expt.	Day	Sta.	NO_2	NO_3	Total N	PO_4	ΔNO_2	ΔNO_3	ΔN	ΔPO_4
I	3	1	0.0107	0.02	0.56	0.0063				
		3	0.0142	0.015	0.0	0.005	+0.0035	-0.005	-0.56	-0.0013
	4	1	0.009	0.0035	0.98	0.0125				
		3	0.011	0.005	1.26	0.0063	+0.002	+0.0015	+0.28	-0.0062
	5	1	0.0054	0.005	0.28	0.0062				
		3	0.0019	0.0035	0.28	0.0	-0.0035	-0.0015	0	-0.0062
J	1	1	0.009	0.0078	0.56	0.005				
		3	0.0054	0.005	0.42	0.001	-0.0036	-0.0028	-0.14	-0.004
	2	1	0.16	0.015	0.56	0.01				
		3	0.0107	0.0	1.96	0.005	-0.14	-0.015	+1.4	-0.005
	3	1	<0.001	0.11	0.84	0.005				
		3	<0.001	0.20	0.84	0.0048	0	+0.09	0	-0.0002
	4	1	0.016	0.02	0.826	0.002				
		3	0.0195	0.0145	1.12	0.0	+0.0035	-0.0065	+0.29	-0.002
	5	1	0.007	0	0.84	0.025				
		3	0.007	0	0.728	0.005	0	0	-0.13	-0.005
K	1	1	0.008	0.001	1.12	0.0				
		3	0.006	0.0037	0.84	0.0	-0.002	+0.0027	-0.28	0
	5	1	0.001	0.0025	1.12	0.001				
		3	0.0018	0.0018	0.84	0.01	+0.0008	-0.0007	-0.28	+0.009
L	1	1	0.0036	0.0095	1.36	0.005				
		3	0.0036	0.0095	1.36	0.005	0	0	0	0
	4	1	0.0142	0.0065	1.68	0.0045				
		3	0.0	0.0040	1.98	0.001	-0.0142	-0.0025	+0.30	-0.0035

TABLE 39 (continued)

Expt.	Day	Sta.	NO_2	NO_3	Total N	PO_4	ΔNO_2	ΔNO_3	ΔN	ΔPO_4
M	1	1	0.009	0.011	0.56	0.001				
		3	0.0019	0.004	0.84	0.005	-0.0071	-0.007	-0.28	+0.004
	5	1	0.0019	0.002	0.84	0.0127				
		3	0.0036	0.0035	0.20	0.02	+0.0017	+0.0015	-0.64	+0.008
N	1	1	0.0	0.003	0.84	0.0065				
		3	0.0019	0.003	0.56	0.001	+0.0019	0	+0.28	+0.0055
	4	1	0.0		0.70	0.0125				
		3	0.0		0.30	0.0063	0		-0.40	-0.0062

DISCUSSION

The Huron River at the channel site, is probably not the most ideal location for a study of this type, due to a lack of diversity of forms. Unfortunately, from various other standpoints, this was the most desirable location available during the study.

The string method appears to be a reasonably reliable method of making quantitative measurements of growth and enumerating the organisms associated with slime buildup. This possesses the advantages of the submerged slide technique but does not appreciably alter the hydraulic characteristics of the channel as does the latter. Of all materials tested in a prototype flume (glass rods, slides, sticks, nets, etc.), this method gave as reproducible results as any. The reproducibility obtained between duplicate dry weights in the channel was on the order of ±5% for samples in the range of 25-100 mg; about 10% in the range 100-300 mg; becoming ±30% in the range 300-1000 mg. Dry weights greater than 1000 mg. varied as much as 50-60% between replicates.

Reproducibility as far as enumeration of the attached organisms is concerned, is of course dependent on several additional factors and would be difficult to assess directly.

The string idea is not new; it has been used by many investigators, but the use of nylon knitting yarn seems to be a novel approach. When viewed microscopically, each of the strands is made up of thousands of individual fibers radiating in all directions, each presenting a potential attachment site. This fine structure contributes the rough feel to the yarn, and because of this structure, the surface area of each 10 cm section is tremendous. Due to the large surface area, the yarn is able to support much more massive growths than a smooth string. For example a 10 cm length of yarn weighing 0.0014 grams can support a wet floc weighing in excess of 30 grams, or about twenty thousand times its own weight!

It is felt that the technique of expressing results as percentage of attached growth per 10 cm of yarn is a valid and useful one. Since the strings afford an equal opportunity for attachment to the incoming biota for 24 hours, their frequency is governed only by current, tolerance and generation time.

Counting becomes very difficult when dealing with massive quantities of Sphaerotilus. It is often necessary to dilute the material several fold to free the associated organisms of their entanglement in the filaments.

As was expected, the influent plankton had little or no relation to the attached population. At one time some consideration was given to maintaining strings in the river as a base-line control. This would not be truly comparable, however, since velocities and flows would be substantially different. It appears that the 24 hour populations before feed began served satisfactorily as controls, since hydraulic conditions were practically identical in the channel. These populations before feed showed no appreciable differences due to variations in velocity, but did respond to changes in temperature and other uncontrollable changes in river conditions.

The chief response of the attached organisms to nutrient addition and/or Sphaerotilus growth was a reduction in frequency of most genera. Following is a list of frequent planktonic and channel forms and their response to nutrient and velocity:

Anabaena circinalis: Common river plankter in August; lacking in July, September and October. Fair channel growth at 10 mg/l and a velocity of 0.68 fps; slight growth at 5 mg/l and a velocity of 1 fps.

Ankistrodesmus sp.: Relatively abundant in early August, present throughout July, August, September and October in river plankton. Excellent channel growth at 10 mg/l and 0.68 fps; slight growth at 5 mg/l and velocities from 0.29 to 0.58 fps.

Aphanizomenon sp.: Common in river plankton in late August through September. Excellent channel growth at 20 mg/l and a velocity of 1 fps and at 10 mg/l and a velocity of 0.25 fps.

Asterionella formosa: Present in river plankton in early October. Excellent channel growth at 10 mg/l and 0.71 fps.

Ceratium hirundinella: Most common river plankter in August through October. Best channel growth at 40 mg/l and a velocity of 1 fps.

Chlorella sp.: Present in river plankton in August and September. Good channel growth at a velocity of 1 fps and a sucrose concentration of 1, 5, 10, and 20 mg/l.

Chlamydomonas sp.: Common river plankter in July, August and September. Fair growth at 1 fps with concentrations of 5, 10, and 20 mg/l.

Closterium sp.: Noted in river plankton in mid-August. Fair channel growth at 20 mg/l and a velocity of 1 fps. Slight growth at all velocities tested at 5 and 10 mg/l.

Cocconeis sp.: Present in river plankton in mid-August. Fair channel growth at 20 mg/l sucrose and 1 fps velocity, and 5 mg/l at 0.76 and 0.5 fps.

Cryptomonas ovata: Present in river plankton throughout August. Fair channel growth at 20 mg/l and 1 fps velocity.

Cyclotella sp.: Not frequently observed in river plankton. Slight channel growth at 5 mg/l sucrose and 1 fps and 10 mg/l with velocities of 0.68 fps and 1 fps.

Cymbella sp.: Observed in river plankton in August. Good channel growth at 5, 10, and 20 mg/l and 1 fps and at 5 mg/l and 0.25 fps to 0.58 fps.

Cymatopleura sp.: Present in river plankton in August. Good channel growth at 5 mg/l sucrose and velocities of 0.56 and 0.68 fps. Fair growth at 5 and 10 mg/l at 0.25 and 0.71 fps respectively.

Diatoma vulgare: Present in river plankton in August and September. Good channel growth at 5, 10, and 20 mg/l and 1 fps and at 5 mg/l at 0.25 fps.

Difflugia sp.: Present in river plankton in July, August and September. Fair channel growth at 10 mg/l sucrose and 0.71 fps.

Euglena sp.: Present in river plankton in August and September. Slight channel growth at all concentrations and velocities tested; best growth at 5 mg/l and 0.76 fps.

Dinobryon sertularia: Present in river plankton in August and October. Fair channel growth at 10 mg/l sucrose and 0.71 fps, or 1 fps.

Desmidium sp.: Present in river plankton in late August. Slight channel growth at 20 and 40 mg/l sucrose and 1 fps; and 5 mg/l at 0.58 fps.

Fragilaria crotonensis: Abundant in river plankton in September and October. Excellent channel growth at 1 mg/l and 1 fps and at 5 mg/l and 0.56 fps, or 0.58 fps.

Gomphosphaeria aponina: In plankton during early and mid-October. Slight growth in channel at 20 and 1 mg/l sucrose and 1 fps; at 10 mg/l sucrose at 0.71 and 0.25 fps; at 5 mg/l and 0.58 fps.

Keratella sp.: Occasionally in river plankton in July through September. Slight channel growth at 40 mg/l sucrose at 1 fps.

Lyngbya sp.: Present in river plankton during October. Good channel growth at 10 mg/l sucrose at 0.68 fps.

Melosira granulata: Dominant river plankter in July through September. Excellent channel growth at 1 mg/l and 5 mg/l at 1 fps and at 10 mg/l sucrose at 0.71 and 0.25 fps.

Melosira varians: Common plankter in August through late October. Excellent channel growth at 5, 10, and 20 mg/l at all velocities except 0.09 fps.

Microspora sp.: Not observed in river plankton. Excellent channel growth at 5 mg/l and 1 fps.

Navicula sp.: Common river plankter in July, August and September. Good channel growth at 1, 5, and 10 mg/l sucrose with velocities from 0.29 to 1 fps. Best growth at 10 mg/l and 1 fps.

Nitzschia sp.: Common river plankter in August and September. Excellent channel growth at 10 mg/l sucrose and 0.71 fps, and 1 fps; slight growth down to 0.29 fps at 5 mg/l.

Oscillatoria sp.: Present in river plankton in October. Excellent channel growth at 5 mg/l with velocities from 0.56 to 0.71 fps. Good growth at all velocities with concentrations of 1 and 10 mg/l.

Pediastrum sp.: Present in river plankton in July through October. Slight channel growth at 10 mg/l sucrose with velocities of 0.71, 0.68 and 0.25 fps and at 5, 20, and 40 mg/l at 1 fps.

Phacus sp.: Present in river plankton in July through September. Good channel growth at 1 mg/l sucrose and 1 fps velocity.

Pleurosigma sp.: Late October river plankter. Good growth at 5 mg/l sucrose with velocities of 0/71 and 0.56 fps.

Rotifers, Unidentified: Found in river plankton in July through October. Good channel growth at 5 mg/l sucrose and 0.56 fps velocity.

Scenedesmus sp.: Present in river plankton in August through October. Fair channel growth at 5, 10 and 20 mg/l sucrose at all velocities tested.

Stauroneis sp.: Rare in river plankton. Good channel growth with 5 mg/l at 1.0 and 0.71 fps.

Stephanodiscus sp.: River plankter during July, August, and early September. Good channel growth at 5 mg/l sucrose with all velocities tested.

Stigeoclonium tenue: Not observed in river plankton. Excellent channel growth at 5 and 10 mg/l at 1 fps.

Surirella sp.: Rare in river plankton. Fair channel growth at 5 mg/l sucrose and 0.56 fps velocity.

Synedra capitata: Rare in river plankton. Fair channel growth at 10 mg/l and 0.68 fps.

Synedra acus: Rare in river plankton. Good channel growth at 5 mg/l sucrose and 0.71 fps velocity.

Tabellaria fenestrata: Present in river plankton intermittently during early August and throughout September. Excellent channel growth at 10 mg/l sucrose and 0.25 fps velocity.

Ulothrix zonata: Not observed in river plankton. Excellent channel growth at 5 and 10 mg/l at 1 fps.

Vorticella sp.: Very rare in river plankton. Growth noted in channel during heavy *Sphaerotilus* growth.

It is likely that some individuals noted were chance associations, but the regularity of appearance of forms such as *Melosira granulata*, *M. varians*, *Navicula*, *Nitzschia*, *Cosmarium*, and *Euglena* in the biological floc, seems to indicate a closer relationship. The exact nature of this relationship would have to be determined through extensive laboratory investigation, beyond the scope of the present work. There is a definite symbiotic relationship between the floc and simuliid and tendipedid larvae and pupae, in that early growths of *Sphaerotilus* were noted to attach first to the mucous and silken strands of these forms, incorporating them as central "stems" in the feathery growth. These macroinvertebrates in turn, were furnished shelter and food by the flocs. This relationship was described by Warren, *et al*. (1964) as having a beneficial effect on trout production in their experimental stream enriched with sucrose. Tendipedid larvae, a favored fish food, were increased tremendously due to the presence of *Sphaerotilus*. The hydrophilid beetles also took advantage of the shelter and food value of the flocs, but it is doubtful that they contributed anything in return. Although these metazoans are primarily algae feeders, they were observed to feed very vigorously on *Sphaerotilus* flocs.

The exact manner in which Sphaerotilus is able to take over and dominate a niche from an established population is still obscure. One observation worthy of note is that in the early hours of nutrient feed, Sphaerotilus became attached to growths of the green alga Spirogyra. Soon, gas bubbles, presumably photosynthetic oxygen, formed between the alga and flocs, but after the alga was blanketed enough to exclude light, gas bubbles formed between the liner and the alga, breaking the latter loose from the attachment and leaving a clean niche, which Sphaerotilus immediately moved in to occupy.

Generally, saturation population occurred in the channel around 72 hours after feed was started. At temperatures of 20°-28°C, heaviest growths were obtained with 5 mg/liter molasses, their magnitude being proportional to velocity, in the range of 1.49 fps to 0.58 fps.

At temperatures below 20°C, saturation population was not attained even after 12 days accumulation. In fact, at temperatures from 9°-17°C, Sphaerotilus was replaced in dominance by a small filamentous bacillus. It is the belief of the writer that a channel of some ten times greater length would be required to obtain saturation population at temperatures of 10°-15°C. This is based in part on the writer's observation of growths in the Altamaha (river) in Georgia over a three year period. With a relatively constant load of modified Kraft process wastes, growths of Sphaerotilus during the winter months extended over some forty miles of river, reaching a maximum around 5 miles below the outfall. At summer temperatures (28°-30°C) however, growth receded to within one mile or less of the outfall, the maximum occurring just below that structure. Unfortunately, observations such as this are not available pertaining to northern streams although, there is a definite need for such information.

The effects of hydraulic characteristics are difficult to assess. It was noted previously that Sphaerotilus flocs do alter these characteristics somewhat. For instance in Experiment N, the channel level at station number one rose from the normal 7-1/8 inches to 11 inches; from 8 inches to 10 inches at station 2; and from 6-1/8 inches to 7-1/2 inches at station 3. This change naturally results in alteration of velocity.

As stated in the Results, there was sufficient nitrogen and phosphorus in the channel at all times to support the biota. Based on these results a total nitrogen concentration of 0.5 mg per liter, and 0.001 mg per liter of ortho-phosphate is sufficient to produce a bloom of Sphaerotilus in the presence of a suitable carbohydrate; in this case 5 mg per liter of molasses. It seems therefore, that most rivers are potential "Sphaerotilus streams" if this carbohydrate be added.

In a series of reaeration experiments, not reported herein, runs were made at 25 and 50 gpm with weir heights from 0.2 ft to 0.8 ft. The k_2/day values ranged from a mean of 7.1 at 25 gpm and 0.6 ft to a mean of 14.8 for 50 gpm and 0.2 ft.; with 14.5 at 0.4 ft, and 14.0 at 0.6 ft. The latter values are in good agreement with the mean k_2/day of 14.6 determined in 1965. Comparing these k_2 values with maximum observed k_c values, which averaged around 21.2, one would expect a much greater DO depression than occurred. This would tend to support the theory of biological extraction and accumulation, (the removal of BOD in excess of that accountable in terms of DO utilized). The observation that maximum nutrient removal, saturation population, and maximum DO utilization seemingly occur in that order, indicates that an equilibrium accumulation is probable. Perhaps BOD removed is not oxidized at the instant of extraction but is transfered from the flowing stream to the biomass and incorporated into the latter by anabolic reactions which do not require oxygen. Kirschner (1966), in pure culture experiments using tagged glucose, concluded that glucose was transformed into the heteropolysaccharide capsule of the organism by pathways not requiring energy from the utilization of oxygen.

Accumulation in these short-run experiments is in terms of active Sphaerotilus growth with equilibrium at saturation population. Accumulation of sloughed growth as sludge (as occurs in streams) did not occur in these experiments. However after the equilibrium accumulation of saturation population is attained, oxygen utilization may exceed BOD removal, due to decomposition within the flocs. This happening is likely responsible for the increased rates noted in the laboratory using the modified reaeration technique, the results of which were reported in

Table 36. It appears probable that Sphaerotilus, upon death, is decomposed more rapidly by the other organisms, than the original substrate. Either that, or Sphaerotilus has enzymatically altered the original substrate, during growth, to a more readily oxidizable form. This seems unlikely because of the short time involved for such alteration. The other possibility, that Sphaerotilus per se utilizes oxygen at a higher rate than the associated organisms, has been disproven repeatedly in laboratory experiments (W.P.—00091—Progress Report—1966).

Further verification of the theory of biological extraction and accumulation depends upon the accuracy of substrate removal rates determined for the channel. It is not felt that either the BOD nor COD tests furnish the required accuracy. The inherent difficulties of the BOD test detract heavily from its usefulness in experimental research, although it is a useful tool to assess the water quality of rivers—the purpose for which it was designed. COD on the other hand, furnishes no information on the biodegradability of the substrate. It is suggested that sucrose determinations in conjunction with total carbon analyses would furnish much more dependable information.

It is suggested that there is a need for further research along the lines of the present work, incorporating the change recommended above. In addition, the use of various types of bottoms (e.g., silt, gravel, rock, etc.) would add considerably more information, since it should support a much more diverse biota at higher trophic levels.

Long term experiments to determine the effects of extended slime buildup seem desirable and one such experiment is planned for the near future. In addition, the channel should lend itself profitably to studies of Sphaerotilus inhibitors.

There is little basis for comparison of the results presented herein with the work of others, because of the differences in methods of approach. The research of both Zimmermann (1961) and Wuhrmann (1964) utilized ground water and sewage at "sommerkalt" temperatures and consequently supported a considerably different biota in their channels.

It should be noted that while the DO-BOD relationships reported herein may be explained as biological extraction, there may be other possible explanations. Major difficulties encountered in the interpretation of these relationships are: the difficulty in determining actual quantities of oxygen utilized by the organisms present; the uncertain role of photosynthesis in the overall oxygen balance; and the limitations of the BOD test.

"BOD removal" could become a more meaningful term if it encompassed a thorough knowledge of the composition of the original substrate, the nature of its alteration by enzymatic action, and the end products involved, as well as the quantity of oxygen utilized. It is felt that such knowledge would shed considerable light into the overall mechanisms of stream self-purification.

CONCLUSIONS

Sphaerotilus natans was stimulated to grow into bloom proportions by addition of 1 mg/liter of sucrose to a natural water containing as little as 0.5 mg/liter of total nitrogen and 0.001 mg/liter of orthophosphate as phosphorus.

The optimum concentration of sucrose for heavy growth of *Sphaerotilus*-dominated flocs in the channel was 5 mg/liter at velocities of 0.58 fps to 1.49 fps in the temperature range of 20°-28°C. Generally, growth was proportional to both temperature and velocity, and classical flocs did not occur in this system below 17°C.

The biological floc community consisted of *Sphaerotilus natans*, the diatoms, *Melosira granulata*, *M. varians*, *Navicula* sp., *Nitzschia* sp.; the desmid, *Cosmarium* sp.; the green euglenoids, *Euglena* sp.; and usually the bacteria-feeding protozoans, *Tetrahymena pyriformis*, *Colpidium colpoda*; occasional amoebae; and often *Bodo* (*caudatum*?) sp. Both tendipedid and simuliid larval forms found a favorable habitat of shelter and food within the biological growths, and the mucous and silken threads of these invertebrates served as formation "nuclei" for *Sphaerotilus* growth.

After receiving nutrient for 72-96 hours, growth in the channel reached a type of equilibrium, at which time, the amount of old growth detaching and floating away just equaled the amount of new growth being formed. The term "saturation population" was proposed to describe this condition. Just prior to this occurrence, BOD removal was at a maximum, without an equivalent utilization of dissolved oxygen. Just after saturation population, the growths were blackened with an odor of hydrogen sulfide, indicating a critical thickness of the floc at which time it was no longer permeable to diffusion of oxygen and/or other gases. When this occurred, BOD removal decreased and DO depression increased, producing a "sag." Samples removed at this time for laboratory rate determinations by a reaeration technique exhibited higher rates of oxygen utilization than samples taken earlier or later. These observations lend support to the theory of biological extraction and accumulation as proposed by Velz and Gannon (1963).

The method of suspending weighted nylon knitting yarn at various points along the channel is a useful and reliable tool for the quantitation of growth, both in enumerating attached organisms, and as an index of dry weight.

SUMMARY

This dissertation describes ecological investigations performed in an outdoor experimental channel receiving Huron River water and beet sugar or crude molasses during two summers of operation (1965-1966).

The channel was assembled in two lengths, 645 feet (1965) and 704 feet (1966), with three or four sampling stations located one near the beginning, one in the middle and one near the end. At each station, three strands of knitting yarn were suspended and allowed to remain 24 hours. After this time, the strands were removed, together with attached growth, and fresh strings replaced. The replicate strings were cut into 10 cm sections, with one half of these sections used for enumeration of attached organisms, while the other half was used for dry weight determinations.

The attached organisms were removed by violently shaking the strings suspended in a measured quantity of water. One drop was then removed and direct microscopic counts and identification made immediately. Dry weights were obtained by drying the strings in aluminum weighing pans overnight at 90°C.

Dissolved oxygen (DO), biochemical oxygen demand (BOD) and nitrogen and phosphorus determinations were also made regularly during the operation. Physical measurements consisted of temperature, velocity and time of passage. Reaeration (k_2) rates and BOD rates (k_c) were calculated from these determinations.

A total of 14 experiments was run, the duration of each being in most cases five days.

In the first series of experiments (A-F), flow through the channel was at 100 gpm at an essentially uniform velocity of 1 fps. Nutrient concentration was varied from 1 mg/liter to 40 mg/liter as sucrose.

In the last series of experiments (G-N), nutrient concentration was held at 5 mg/liter and velocity varied at the three stations by altering the height of an overflow weir between 0.0 and 0.6 ft., and changing the flow between 25 gpm and 200 gpm. This provided a range of velocities at the stations from 0.09 fps to 1.49 fps.

The results of attached organism determinations are presented for each station in detail following each experiment and their response to nutrient concentration and velocity summarized in the Discussion.

Sphaerotilus-dominated biological flocs were stimulated to bloom proportions within 30 hours after the addition of as little as 1 mg/liter of sucrose. Maximum growth was obtained at a concentration of 5 mg/liter at velocities from 0.58 to 1.49 fps in the temperature range of 20°-28°C after around 72 hours of feeding. About this time, detaching and floating material was equivalent to new material being formed, and the term "saturation population" was proposed to describe this condition.

The biological floc community was composed chiefly of Sphaerotilus natans, Melosira varians, M. granulata, Nitzschia sp., Navicula sp., *Cosmarium* sp., *Euglena* sp., and the protozoans, *Bodo* sp., *Tetrahymena pyriformis*, *Colpidium colpoda*, and *Amoeba* sp. Tendipedid and simuliid larval forms also apparently found a mutualistic association within the flocs.

Just at or prior to saturation population being attained, BOD removal was at a maximum, while just afterwards, DO depression reached a maximum. Evidence is presented to substantiate the biological extraction-accumulation theory of Velz and Gannon (1963).

Saturation population and classical *Sphaerotilus* flocs could not be obtained at temperatures below 17°C. The typical form was replaced in dominance at low temperatures by an unidentified filamentous bacillus, the total mass of which never approached that of the classical growth.

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SECTION V

REAERATION AND PHOTOSYNTHETIC OXYGEN RELATIONSHIPS IN EXPERIMENTAL CHANNELS

by
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Department of Environmental Health

INTRODUCTION

The classical oxygen sag relationship first proposed by H. W. Streeter and E. B. Phelps(1) in 1925 has proven valuable in evaluating the influence of organic pollution on the dissolved oxygen assets of a stream. The assumption is made that two major processes are occurring: (1) biochemical oxidation of the organic material by bacterial action; and (2) reaeration of river water from the atmosphere. It is, of course, recognized that several additional sources of oxygen supply and demand contribute to river oxygen balance as listed by Dobbins.(2)

Over the years considerable interest has existed in developing an accurate evaluation of reaeration from the atmosphere, with lack of agreement as to the best formulation for such evaluation. Because of this and because of the previous need to evaluate the reaeration potential of natural waters in the artificial channel, interest has developed in the subject, resulting in the present studies involving exploration of reaeration of natural waters from a fundamental standpoint using the channel.

Another influence on river oxygen balance which has been appreciated but which has received little attention until recently has been the metabolic activities of aquatic plants. Oxygen is produced as a result of photosynthetic activity, while some of this oxygen is required by the plants to meet their continuous respiratory needs. In many cases there is an excess of oxygen which is then contributed to the dissolved oxygen assets of the water. This is another study area which has been stimulated by earlier work, where it appears the channel will be a useful tool in gaining some fundamental insight into the role of photosynthesis in oxygen balance of natural waters.

ATMOSPHERIC REAERATION

The transfer of oxygen from the atmosphere to the bulk liquid can only occur across the interfacial area separating the two phases. Once the surface layer of liquid has absorbed oxygen and the oxygen deficit from saturation has been decreased, the driving force is thus decreased and the oxygen transfer process decreases also, even though the bulk of the liquid may still be wholly unsaturated.

In a completely quiescient body of water, the removal of this liquid "cap" can only occur by a) diffusion of oxygen molecules from the "cap" into the bulk, and b) by streaming due to density differences in the liquid "cap" and the bulk, caused by cooling at the surface due to evaporation.

In a completely turbulent body of water, the removal of the "cap" is almost instantaneous and continuous; thus the oxygen deficit of the bulk decreases uniformly.

In a moving body of water such as a stream, the condition is somewhere between the two above mentioned extremes. It is thus apparent that the rate of reaeration in a flowing stream is controlled by surface renewal which in turn is controlled by the various stream parameters influencing surface renewal, such as (a) depth, (b) longitudinal velocity, (c) geometric shape of the cross-section, and (d) degree of turbulence which is influenced by the forementioned parameters in addition to bottom roughness and viscosity.

Several approaches have been presented over the years to define the mechanism of atmospheric reaeration. One of the earliest and most generally used, yet most controversial, explanation of the absorption phenomenon was advanced in 1924 by Whitman and Lewis, "The Two-Film Theory."(3) A similar approach was also advanced by Adeney and Becker.(4) These theories require, among other things, the physical existence of a laminar liquid film at the liquid interface.

In 1930, Miyamato(5) advanced "The Kinetic Theory of Gas Absorption." In this theory, the hydrodynamic factors seemed to have been neglected, especially turbulence.

Then "The Penetration Theory" was introduced by Higbie.(6) This theory assumes that renewable surface layers are instantaneously and continuously saturated with the solute gas.

Danakwerts(7) advanced a "Surface Renewal Theory" where a surface "age" is independent of the residence time at the surface. This theory seems to be a very rational one.

"The Surface Rejuvenation Theory" by Kishinevski(8) assumes that the surface layer becomes completely and instantaneously saturated and that the absorption process is controlled entirely by turbulent diffusion.

These are the classical approaches of defining the controlling mechanisms of gas absorption. With the advances in hydrodynamics, several approaches have been presented to evaluate the reoxygenation coefficient, k_2, from measurable hydraulic stream parameters.

Since the presentation of the Streeter-Phelps equation in 1925, the common practice in determining the k_2 value of a stream has been to evaluate all other terms of the equation and then to solve for k_2.

One of the first attempts to predict the reaeration coefficient from basic hydraulic data was presented by O'Connor and Dobbins(9) in 1956, namely:

$$k_2 = \frac{480\ Dm^{1/2}\ Se^{1/4}}{h^{3/4}} \qquad \text{(non-isotropic flow)}$$

$$k_2 = \frac{Dm\ U}{2.31\ h^{3/2}} \qquad \text{(isotropic flow)}$$

In 1960, Krenkel(10) presented another approach in his Ph.d. thesis at the University of California. He utilized the longitudinal mixing coefficient, D_L, as a measure of the turbulent intensity. His equation is:

$$k_2 = 4.3(10)^{-5} D_L^{-1.15} (h)^{-1.92}$$

In 1962, Krenkel and Orlob(11) revised Krenkel's original equation as:

$$k_2 = 1.42(e)^{Ea/RT} \frac{D_L}{h^2} - 0.0069$$

Churchill, Elmore, and Buckingham(12) presented an equation in 1962 on river data from the TVA rivers. It is:

$$k_2 = 5.026(U)^{-0.969} (h)^{-1.673}$$

In 1965 a report by Tsivoglou, et al(13) introduced a theory of using radioactive gases, initially Krypton and Radon, as tracers to simulate the process of oxygen pickup or release under turbulent conditions. The report is only for the initial laboratory phase, but the results indicate the proposed theory may have merit as a method of relating the transfer process of a radioactive gas to that of oxygen and to the dispersion characteristics of a nongaseous tracer under turbulent conditions. In 1968 Tsivoglou, et al(14) described the use of tracer measurement of stream reaeration for the Jackson River between Covington and Clifton Forge, Virginia, demonstrating that independent and accurate direct field evaluation of stream reaeration capacity by the use of tracers is feasible and practical, and that the results that are obtained are quite reproductible.

In 1965 Dobbins(15) proposed a new theory based on the Kolmogoroff similarity principle, namely:

$$k_2 = \frac{0.12\, C_A\, A\, E^{3/8} \coth\left[BE^{1/8}/C_4{}^{1/2}\right]}{C_4^{3/2}\, h}$$

These proposed approaches, and numerous others, have been found to predict reaeration rates accurately in some situations, but more often than not, inaccurate predictions result when used outside the area of their formulation. A recent study by Thackston and Krenkel(16) indicated the fallacy in many of the equations. They concluded that much additional work is required in this area, especially relating to field investigations.

Based on this literature review, a study has been proposed to investigate the effect of turbulence on reoxygenation in the outdoor, artificial open channel. The approach will consist of utilization of (a) the longitudinal mixing coefficient, D_L, and (b) the bottom roughness of the channel as measured by Mannings' "n" factor and/or the shear velocity, $\overset{*}{U}$.

Incorporating these measurements of the effect of turbulence on surface renewal and thus reoxygenation, it is the intent of the proposed study to answer the following questions:

(a) Can a method for predicting D_L from various hydraulic parameters be developed for a wide range of channel conditions?

(b) Can the theoretical Manning's roughness coefficient and the shear velocity describe, accurately and quantitatively, the effect of turbulence, and thus surface renewal, or reoxygenation?

(c) Can the effect on reoxygenation of the channel roughness, which increases the depth and decreases the mean longitudinal velocity with increased roughness, be expressed by this change in depth and velocity or must the effect of this roughness on reaeration be accounted for in a parameter expressing the vertical eddy diffusion, which is proportional to the longitudinal eddy diffusion?

(d) Can the rate of reaeration be predicted from a calculated or observed D_L in the channel?

(e) If significant relationships between reoxygenation and D_L and/or $\overset{*}{U}$ or n can be established, how sensitive is the rate of reoxygenation to changes in these parameters?

PHOTOSYNTHETIC OXYGEN PRODUCTION

In recent years the efforts of many laboratories have been directed towards studies concerning the photosynthetic capacity of algae and higher plants. Such studies are descendents of the initial observations of Priestley in 1771 that green plants in some way "freshen" the air in which they grow. Though modern experimental technique has become more sophisticated over the past decades, some of Priestley's puzzle is as perplexing now as it was then.

Although the complexity of the photosynthetic process has stimulated and now taxes some of the best researchers of our time, Priestley's inference that green plants can improve or "freshen" an environment has received only general recognition as the obvious result of oxygen evolution. Ecologists were the first experimenters to evaluate photosynthesis in terms of productivity, temperature, light intensity, and nutrient availability.

A considerable part of this scientific effort has been expended in developing methods of measuring energy flow in communities, Odum (1956;57)(17,18); Verduin (1957)(19); and others. These methods of determining production and/or photosynthesis and respiration range from dark and light bottle experiments to the more sophisticated diurnal curve method.

Odum (1956;57) calculated the productivity of thermal springs from diurnal changes in dissolved oxygen concentration, employing corrections for diffusion of oxygen through the surface. Similar methods have been used by Edwards (1962)(20) and Owens (1965)(21) for temperate rivers, and McConnell and Sigler (1959)(22) who attempted to determine the productivity of a mountain stream.

It was from these studies of photosynthesis and productivity that the researchers proposed equations of theoretical considerations of daily process of oxygen metabolism in flowing waters. The relationship of respiration to photosynthesis may be stated as:

$$Q = P - R \pm D$$

Where:

Q = rate of change of dissolved oxygen per area
P = rate of gross primary production per area
R = rate of respiration per area
D = rate of uptake of oxygen or its rate of loss by diffusion per area (depending upon whether the water is under-saturated or super-saturated with oxygen with respect to air).

During the hours of darkness, the rate of change of dissolved oxygen content of the water is determined only by rates of community respiration and diffusion through the water surface.

$$Q = D - R$$

and the rate of diffusion (D), depends upon the degree of saturation of the water:

$$D = f(C_s - C) \quad ,$$

Where:

f = the exchange coefficient of the reach
C_s = the average saturation concentration within the reach
C = the average dissolved oxygen

by substituting for D, $f(C_s - C)$, it is possible to calculate the exchange coefficient (f) and the respiration rate (R);

$$Q = f(C_s - C) - R$$

if one assumes that the community respiration is constant, Odum (1956;57), or that it varies in a predictable fashion with temperature and oxygen concentration, Owens and Edwards (1962).

By measuring the rate of growth of an aquatic plant community and assuming given photosynthetic and respiratory quotients, it is possible to estimate the net amount of oxygen released. Oxygen produced by photosynthesis per se was not considered in the original Streeter-Phelps (1925)(1) equation for oxygen balance in polluted streams. Camp's (1965)(23) study on the Merrimack River in Massachusetts indicated that atmospheric reaeration is relatively insignificant as compared to the dissolved oxygen produced by photosynthesis. While O'Connell and Thomas (1965)(24) stated that in flowing water where the oxygen production is a result of benthic algae and rooted aquatic plants rather than phytoplankton, this phenomenon will invariably be found to be a definite liability to the stream's oxygen balance rather than an asset. When other researchers like Dobbins (1964)(2) and O'Connor (1966)(25) evaluated the sag equation, photosynthesis was given only slight consideration. Whether this additional oxygen should be considered as contributing to the usable oxygen assets of the stream remains a controversial subject.

The main purpose of this research is to investigate the role of photosynthesis (P) in the overall oxygen balance of natural water, using experimental channels. For many years photosynthesis has been recognized as a factor in oxygen balance studies, but generally has been ignored or assumed to balance respiration (R).

The major hypothesis to be tested is that oxygen produced by photosynthesis is a definable oxygen asset in flowing natural waters.

A second aim is to determine the oxygen production attributable to both phytoplankton and periphyton communities under natural conditions in relation to various oxygen deficits.

A third aim would be improvement and development of appropriate procedures and instrumentation.

A fourth and final aim would be an evaluation of the photosynthesis factor for incorporation in existing extensions of the oxygen sag equation for a more precise method of forecasting dissolved oxygen levels.

A knowledge of the variation in P - R, the net oxygen change caused by all aquatic inhabitants, taking into consideration the combined and separate effects of the attached and free-floating organisms, would be of great value in forecasting dissolved oxygen (D.O.) levels for waste loading purposes. It is hoped that the proposed research will provide not only this information, but in addition, needed insight into the numbers and types of organisms involved.

MATERIALS AND METHODS

The experimental channel used for this study is located outdoors on the grounds of the Ann Arbor Waste Water Treatment Plant. It has an adjustable cross-section and slope, and has been previously described in detail by Gannon, et al.(26) It has a length of 704 ft, a width of approximately 1.4 ft, and a depth of approximately 0.8 ft, with the general layout used in 1967 illustrated in Figure 1 and the cross-sectional shape shown in Figure 2.

A 7,000 gal storage reservoir as shown in Figure 5 is used to store the water so that a constant water quality can be maintained. Six pumps rated at 110 G.P.M. each provide enough flexibility of flow so as to completely cover all the flow ranges the channel is capable of handling.

The dissolved oxygen was continuously measured at the inlet and outlet by Beckman dissolved oxygen analyzers shown in Figure 4. They were connected to Gelman continuously recording strip chart recorders. At two intermediate stations, the dissolved oxygen was continuously recorded with Gelman galvanic cells connected to strip chart recorders. At all stations the temperature was recorded each time samples were taken by use of 0-50°C thermometers. The pH was continuously recorded at the outlet by a Beckman pH meter shown in Figure 6.

Samples for dissolved oxygen were taken by use of a syphon tube and BOD bottles; the same method was used to collect biological and suspended solids samples.

The Winkler dissolved oxygen samples were fixed at the stations and titrated in the field laboratory, Figure 3, with a normalized solution of sodium thiosulfate, and starch as the indicator. The biochemical oxygen demand was determined on incubated samples at 20°C. Settleable solids were determined by use of three Imhoff Cones. Total solids and suspended solids were also determined according to Standard Methods.(27) Biological tests for the presence of algae consisted of microscopic examination and a 24 hr light and dark bottle test for oxygen balances.

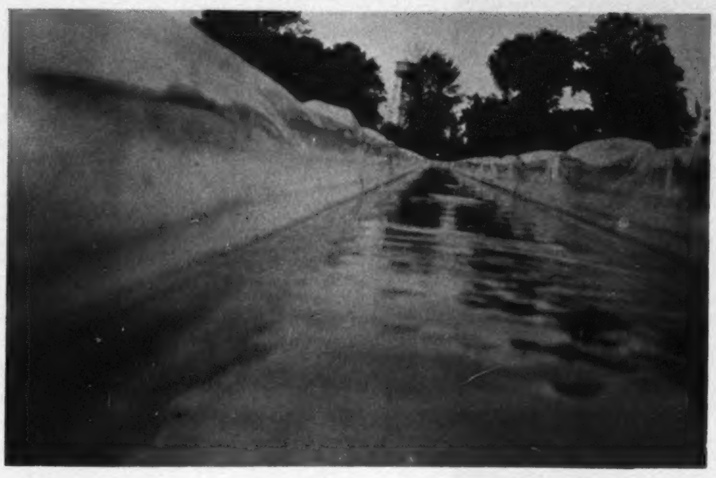


Figure 16. Water surface at 100 gpm—
no channel obstructions.

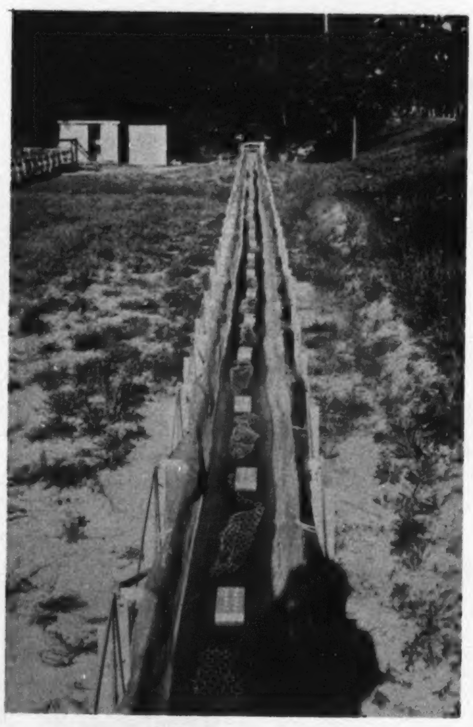


Figure 17. Brick laid lengthwise
on chicken fencing.

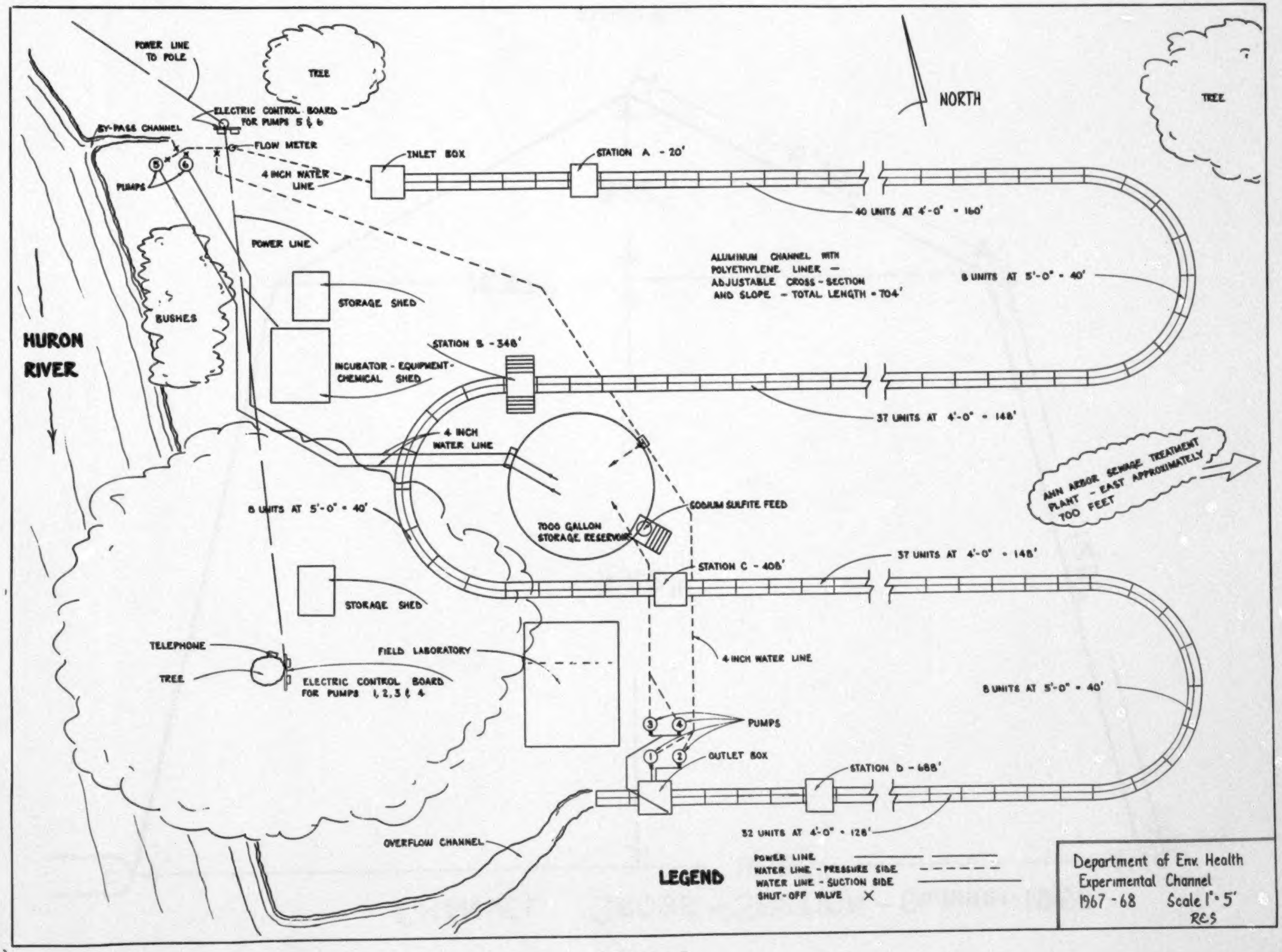

POWER LINE TO POLE
TREE
ELECTRIC CONTROL BOARD FOR PUMPS 5 & 6
BY-PASS CHANNEL
FLOW METER
PUMPS
5
6
4 INCH WATER LINE
INLET BOX
STATION A - 20'
NORTH
TREE
40 UNITS AT 4'-0" = 160'
POWER LINE
BUSHES
HURON RIVER
STORAGE SHED
ALUMINUM CHANNEL WITH POLYETHYLENE LINER — ADJUSTABLE CROSS-SECTION AND SLOPE - TOTAL LENGTH = 704'
8 UNITS AT 5'-0" = 40'
STATION B - 348'
INCUBATOR - EQUIPMENT - CHEMICAL SHED
37 UNITS AT 4'-0" = 148'
4 INCH WATER LINE
ANN ARBOR SEWAGE TREATMENT PLANT - EAST APPROXIMATELY 700 FEET
8 UNITS AT 5'-0" = 40'
7000 GALLON STORAGE RESERVOIR
SODIUM SULFITE FEED
37 UNITS AT 4'-0" = 148'
STATION C - 408'
STORAGE SHED
TELEPHONE
TREE
FIELD LABORATORY
ELECTRIC CONTROL BOARD FOR PUMPS 1, 2, 3 & 4
4 INCH WATER LINE
8 UNITS AT 5'-0" = 40'
PUMPS
3
4
1
2
OUTLET BOX
STATION D - 688'
32 UNITS AT 4'-0" = 128'
OVERFLOW CHANNEL
LEGEND
POWER LINE
WATER LINE - PRESSURE SIDE
WATER LINE - SUCTION SIDE
SHUT-OFF VALVE X
Department of Env. Health
Experimental Channel
1967 - 68 Scale 1" = 5'
RES

Figure 1

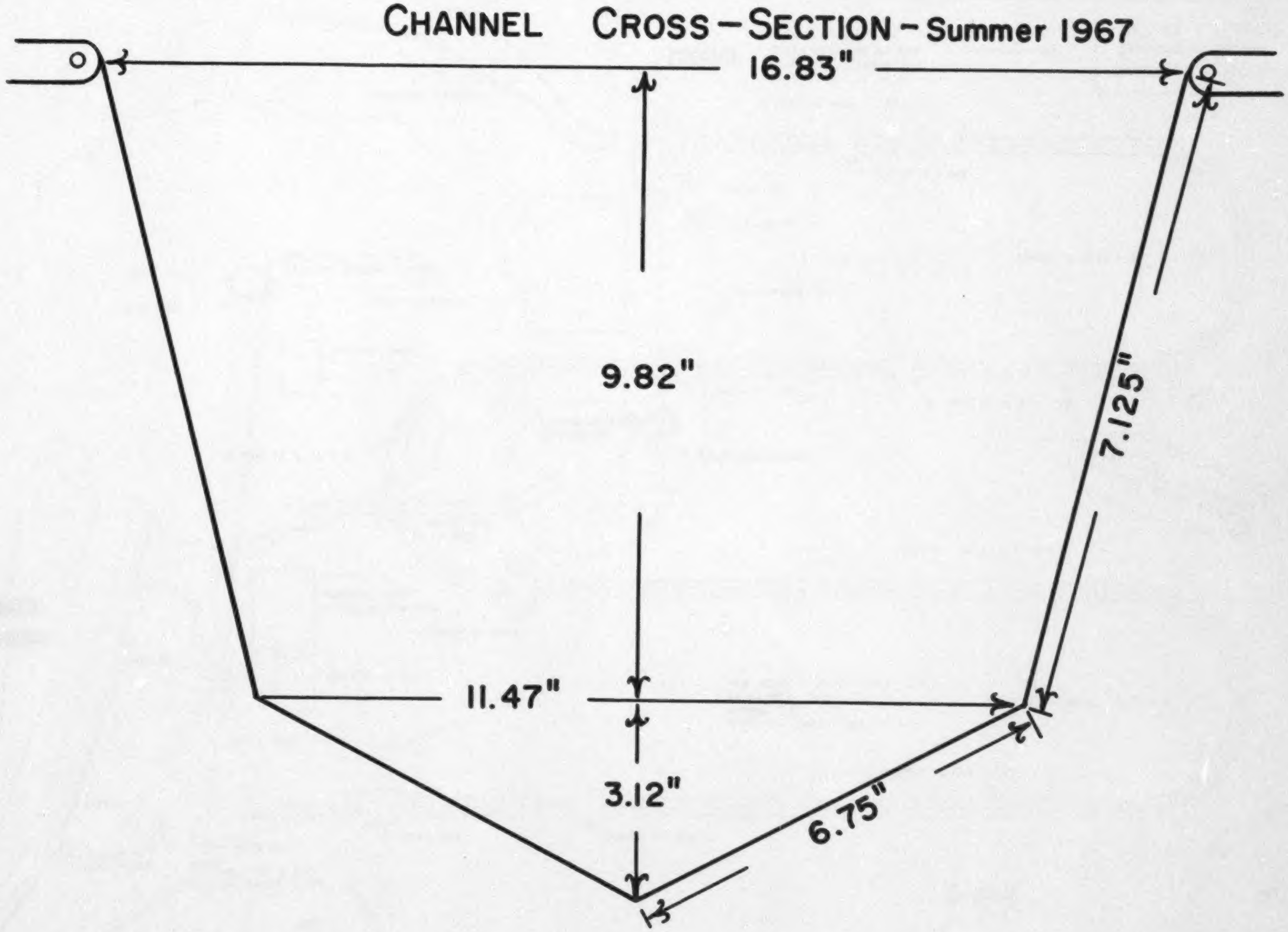

CHANNEL CROSS-SECTION - Summer 1967
16.83"
9.82"
7.125"
11.47"
3.12"
6.75"

Figure 2

EXPERIMENTAL OUTDOOR CHANNEL

Selected Views

Summer, 1967

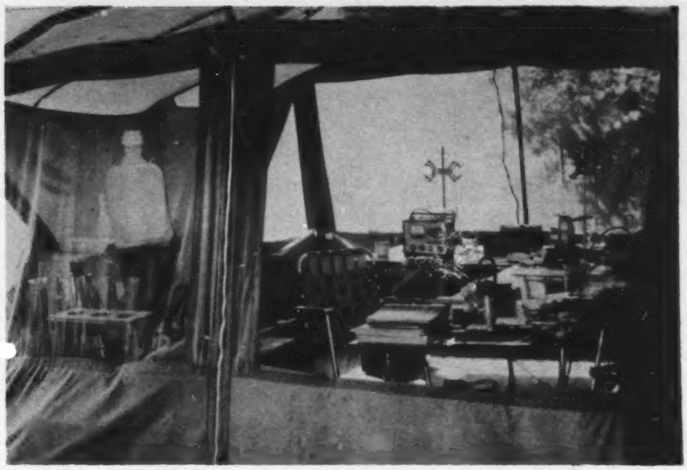


Figure 3. Field laboratory.

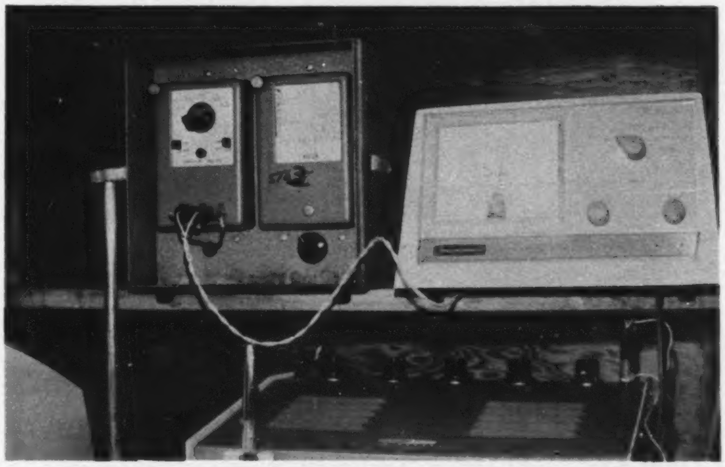


Figure 4. Beckman dissolved oxygen analyzer and strip chart recorder.

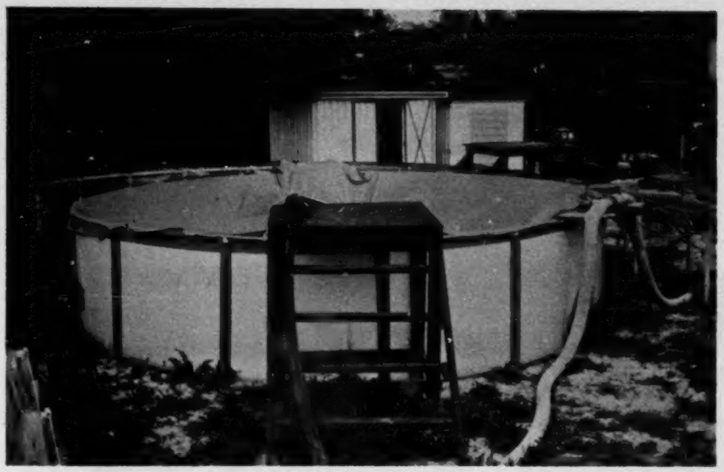


Figure 5. 7000 gallon storage reservoir.

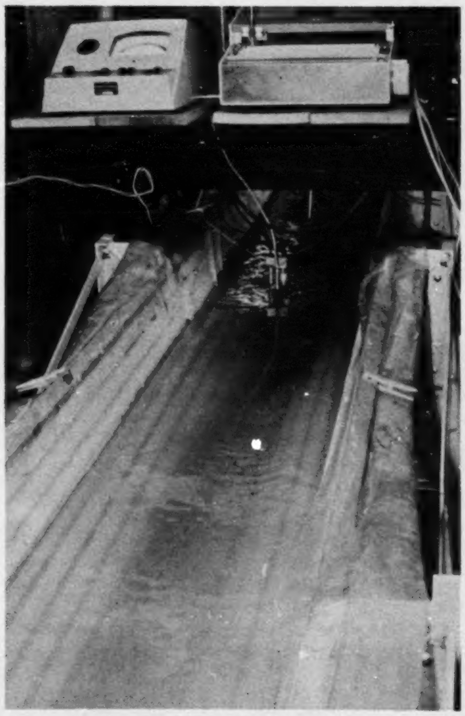


Figure 6. Recording probes for D.O. and flow-through measurement.

ATMOSPHERIC REAERATION

The water in the storage reservoir was treated with copper sulfate and chlorine to kill all biological forms of life. Samples were examined under the microscope for biological growth; light and dark bottle test were also performed. At no time was significant biological activity found to be present after such treatment. The orthotolidine test was used to determine the chlorine residual before each day's run. The chlorine used was hypochlorite from bleach.

Beckman dissolved oxygen analyzers, or galvanic cells, Figure 4, were checked against the modified Winkler dissolved oxygen test. The time of passage was determined by (a) flow meter readings and knowledge of the cross-sectional area, and (b) flow-through curves of slugs of dilute H_2SO_4, NaOH, and depleted D.O. slugs of water. These results were compared, and the D.O. depression curve finally was incorporated as the best method. A constant feed pump was installed near the storage reservoir to deliver a steady solution of Na_2SO_3 into the reservoir water, which contained a colbalt catalyst, thus maintaining a depleted dissolved oxygen level. Rolls of chicken fencing were cut and rolled into small rolls to act as roughness in the channel; also, building bricks were obtained for the same purpose. Any one batch of water was utilized for no longer than two weeks before it was replaced.

The experimental runs consisted of varying flows with various channel roughness present. These runs were:

(1) Flows of 75-250 gpm with no induced channel roughness
(2) Flows of 75-200 gpm with chicken fencing present
(3) Flows of 75-250 gpm with brick laid length-wise on the chicken wire
(4) Flows of 75-175 gpm with brick laid, width-wise, broad side down on the chicken wire
(5) Flows of 125-275 gpm with brick laid width-wise, broad side up, on the chicken wire
(6) Flows of 75-175 gpm with only bricks laid length-wise
(7) Flows of 125-175 gpm with only bricks laid width-wise, broad side down
(8) Flows of 175 gpm with only bricks laid width-wise, broad side up.

Once the flow rate was set for any one run, the width of water surface and the depth were measured at six points, each 8 ft apart, for each station. These were averaged and recorded as the depth and width at that station. This was checked against the theoretical time of passage from the formula:

$$\left(\text{Velocity} = \frac{\text{Discharge}}{\text{Cross Sectional Area}}\right)$$

The instruments at each station were standardized using the Winkler test. At various periods of time, samples were taken at the inlet station and the recording chart was simultaneously marked to insure proper recording. The samples obtained at the remaining stations were from the same theoretical slug of water, since the sampling time was the time of passage from the inlet station to the station in question.

The samples were obtained with a syphon requiring approximately 20 sec to fill the BOD sampling bottle. At the time of sampling the water temperature was recorded. During the experiment, samples were obtained and light and dark bottle tests were performed as was a test for solids.

The dissolved oxygen level was kept depressed by a constant feed of sodium sulfite solution into the reservoir; the water contained approximately 1.0 ppm of cobalt catalyst to ensure rapid deoxygenation of the water.

Photographic documentation was made of all significant conditions and several illustrations are included later in this report.

PHOTOSYNTHETIC OXYGEN PRODUCTION

To the previously mentioned laboratory instrumentation and procedures used for the atmospheric reaeration studies, additional equipment was needed to carry out determination of oxygen produced by photosynthesis. An International Centrifuge Model CS was used to concentrate both the plankton and periphyton samples. The total volume that can be processed at one time is 400 ml. The automatic timer can be set from 0 to 120 min and revolutions of 6,000 per min can be achieved.

Two microscopes were used in the qualitative and quantitative enumeration of organisms. One, a compound microscope with camera attachment, was used for the identification of some of the cleaned diatom populations that were prevalent in both the plankton and periphyton samples. Photomicrographs were freely taken for taxonomic identification if confirmation was necessary. The microscope was equipped with 10 power oculars and 3 nose pieces 10x, 43x, and 97x.

The first series of experiments dealt with physical reaeration where the water was poisoned by appropriate chemicals to destroy both photosynthetic and bacterial activity. The same procedure as described previously was used in the photosynthetic phase to collect and determine D.O., B.O.D., pH, temperature, time of passage, and light and dark bottle data, except that the experiments were carried out with river water that contained normal river fauna.

While the channel was being utilized with the atmospheric reaeration experiments, preliminary trials were carried out in the laboratory to determine sample size and its analysis for organisms.

It was determined that a 250 ml grab sample was statistically large enough to give a good representation of the organisms that make up the aquatic community. The sampling method for phytoplankton utilized in this study is what is referred to by Lackey(28) as a "dip sample." Water samples, taken unconcentrated from the intake box of the channel, were centrifuged in the International Centrifuge Model CS for 10 min at 2,000 rpm. From previous work, it was felt that the time and revolutions were of sufficient magnitude to concentrate almost all of the organisms that were present in the sample. The supernatent was almost entirely removed leaving only about 10 ml of the concentrate. The organisms thus concentrated were then examined alive under the microscope under low and high power for qualitative analysis.

The organisms enumerated in the study included those defined as plankton by Ingram and Palmer.(29)

> The term plankton...refers to those minute unattached animals and plants, exclusive of bacteria, that require at least the standard low power of a compound microscope for identification. For practical purposes the term includes, in addition, the microorganisms which develop originally as attached forms but break loose to become a part of the drifting population. Among the plankton are certain algae—*Aphanizomenon*, *Volvox*, *Anabaena*, *Microcystis* (*Polycystis*), *Dinobryon*, *Synerdra*, *Synura*, *Asterionella*, and animals that may be associated with them—*Cyclops*, *Daphnia*, *Bosmina*, *Hydra*, and *Planaria*.

The counting procedure used to enumerate these organisms is a drop sedimentation method described by Lackey.(28) After the dip sample was concentrated by centrifuging, the concentrate was measured in terms of drops after the removal of most of the supernanent. The number of drops, then, has a definite relationship to the amount centrifuged. After the measurement was made, the concentrate was suspended in a small known volume and a drop of the agitated sample was transferred from a pipette to a slide. The orifice of the pipette was such that it delivered 15 drops per ml. The drop was then covered with a No. 1 cover slip. For enumeration, two passes entirely across the coverslide, each of which was at right angles to the other and passes through the center, were examined under low power (100 magnification). For the microscope used, one pass represented 1/22 of an entire drop. Eleven drops per sample were examined with two passes per drop, yielding a total of examination of 22 passes per sample or, in essence, one entire drop was counted. The following formula expresses the relationship between the number or organisms per ml. Thus,

No. Organisms/pass x 22 passes/drop x 15 drops/ml = No. of organisms/ml

The phytoplankton fauna was determined by the use of the grab sample technique as described earlier in this paper. The periphyton was harvested from two different sampling substrates. The first method consisted of 1 in. square piece of plastic liner attached to a stiff wire, which was supported and introduced from the top of the channel by an aluminum bar as shown in Figure 7. The other method utilized a slide rack which contained regular microscope slides to which a one inch square piece of plastic liner was glued as shown in Figure 8. Figure 9 illustrates the position of these sampling methods in the channel. At specific intervals, these pieces of plastic with the attached growth were carefully

EXPERIMENTAL OUTDOOR CHANNEL
SUMMER, 1967
PERIPHYTON ATTACHMENT SITES

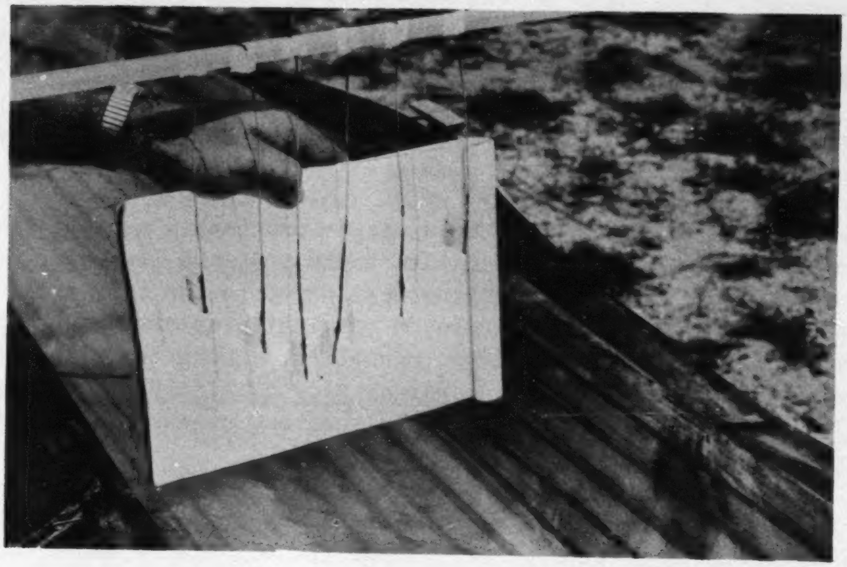


Figure 7

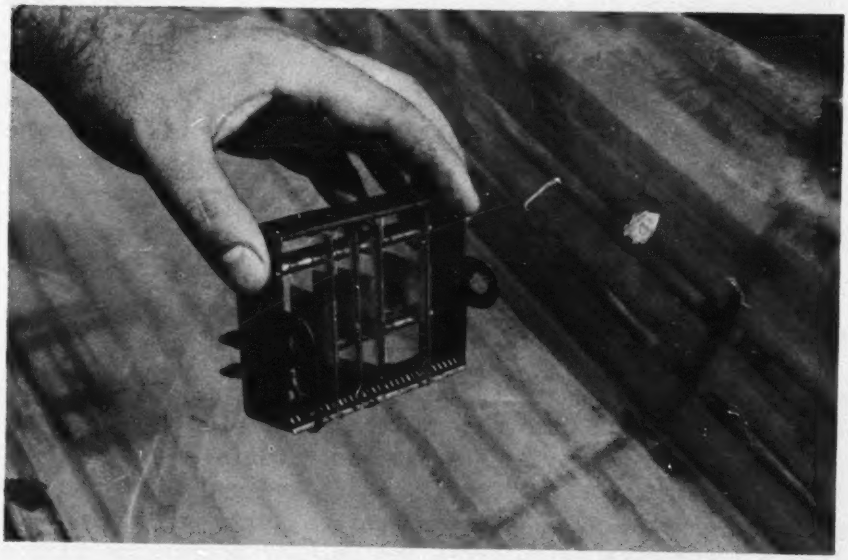


Figure 8

EXPERIMENTAL OUTDOOR CHANNEL

SUMMER, 1967

Both sampling methods which
are employed to determine periphyton
production at 100 Gal. per minute

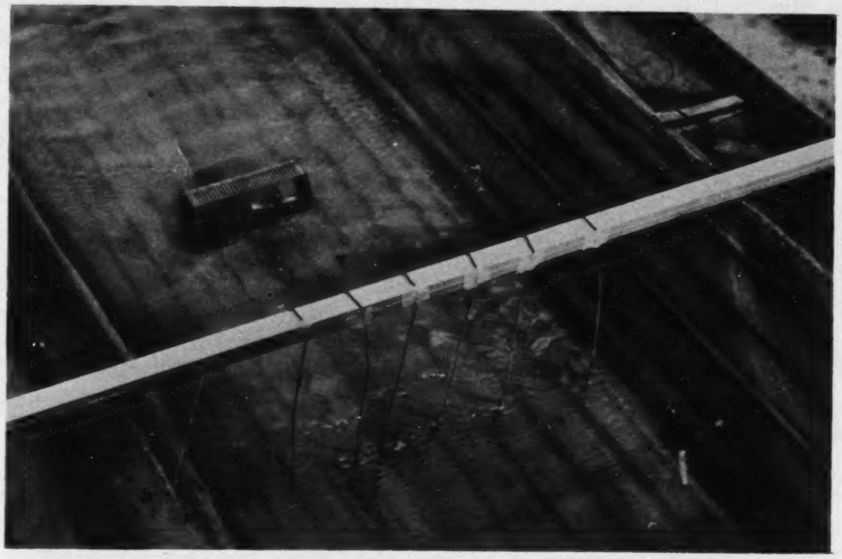


Figure 9

removed to a 50 ml vial and suspended with 20 ml of distilled water. The same procedure for qualitative enumeration was followed as described earlier.

EXPERIMENTAL RESULTS

ATMOSPHERIC REAERATION

Several illustrations of various flow and turbulence conditions are presented. Figure 10 shows the run at 75 gpm with only the rolls of chicken fencing present; the same flow with only the brick present is shown in Figure 11. Figures 12 and 13 show a flow of 100 gpm at station A and B, respectively with no induced roughness present. These same flows, but with brick and fencing both present are shown in Figures 14 and 15. Figure 16 is a surface view of the conditions shown in Figures 12 and 13. Figure 17 shows the arrangement of brick on fencing used throughout the runs. The inlet condition of a flow of 150 gpm with both brick and fence is shown in Figure 18. These same conditions at station B are shown in Figure 19.

Included in Tables 1 and 2 is a list of each of the 34 runs made in the summer of 1967. It indicates the flow rate, average longitudinal velocity, time of passage, average width, existing channel conditions and the oxygen pick-ups per unit surface area for several oxygen deficit levels at each run.

The depths were taken at 24 locations throughout the channel for each run, as were the channel widths; the velocities were computed from each of these cross-sections, averaged and compared to the flow-through curve velocity using the mode. The temperatures were periodically taken at 4 locations throughout the runs. The dissolved oxygen deficit varied over a large range during a run and random selections were chosen for the Table 2 list. The flow-through curve for each run was recorded either by periodic readings or by a continuous recording on a strip chart recorder.

During the runs, samples were also taken to determine (a) total solids present, settleable and suspended, (b) light and dark bottle test, and (c) 5-day, 20°C B.O.D. test.

The establishment of four sampling stations, A, B, C, and D at 20', 344', 408', and 668' from the inlet, respectively, will allow for investigation of the progressive change in oxygen pick-up as the water moves through the channel. Throughout the passage of a slug of water, the most important influence on the rate of oxygen pick-up is the renewal of the surface, which is directly influenced by the vertical velocities. Two important conditions are responsible for the vertical currents: (a) the bottom roughness which diverts longitudinal eddies upward toward the vertical direction and the surface, and (b) the velocity profile which causes upward eddies from slower moving layers of water beneath it.

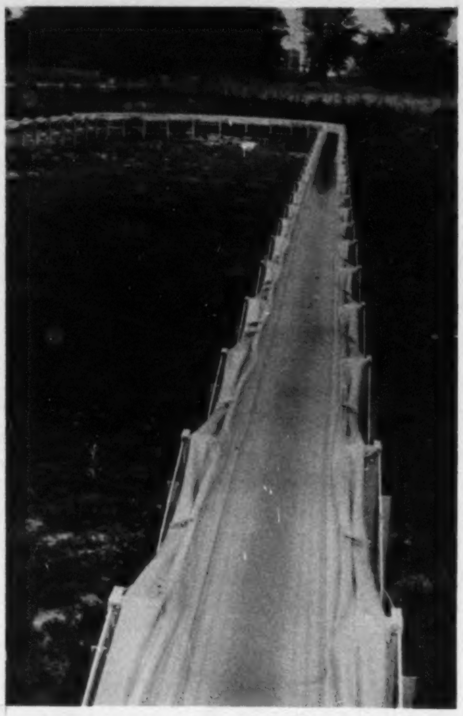
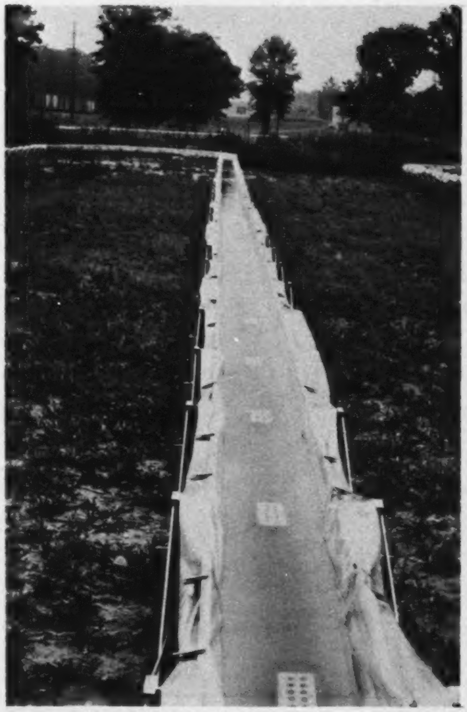


Figure 10. Flow at 75 gpm—fence present (324 ft from inlet—upstream).

Figure 11. Flow at 75 gpm—brick present (324 ft from inlet—upstream).



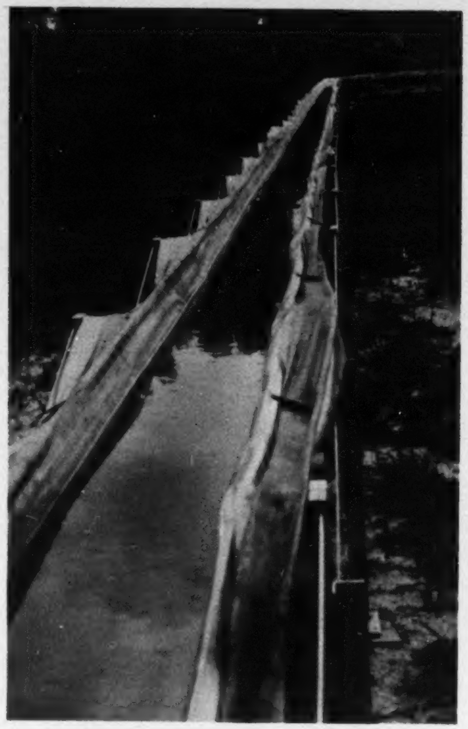
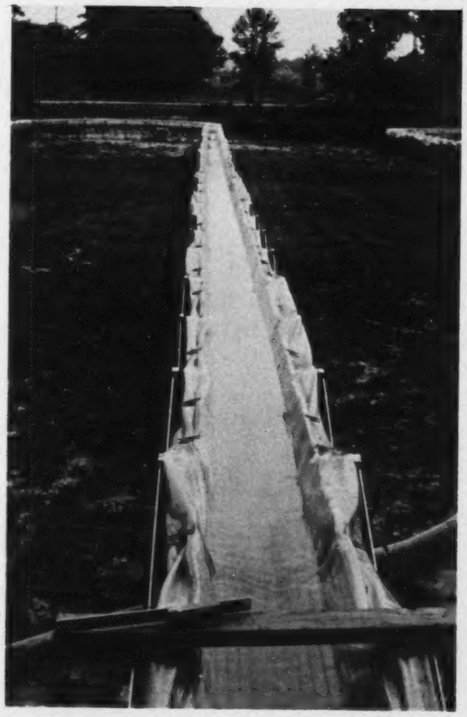


Figure 12. Flow at 100 gpm—no channel obstructions (25 ft from inlet—downstream).

Figure 13. Flow at 100 gpm—no channel obstructions (324 ft from inlet—upstream).



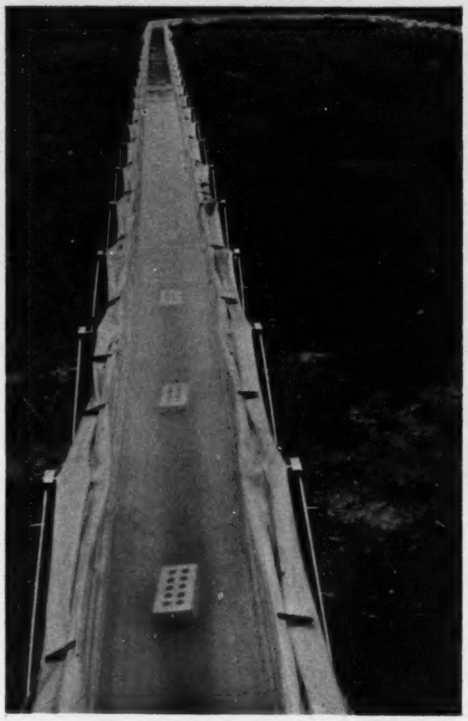
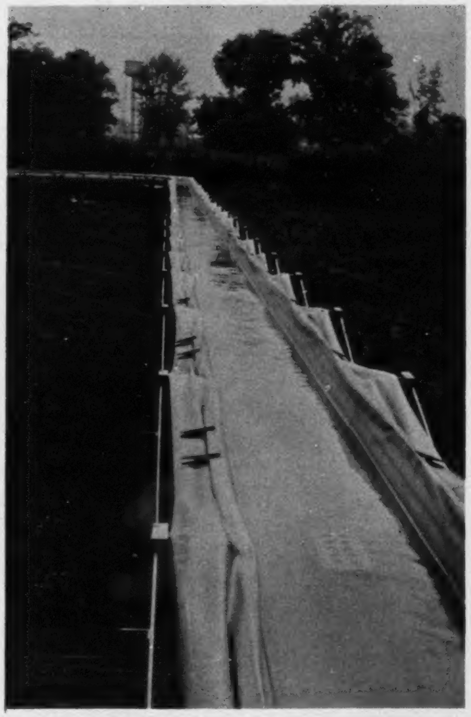


Figure 14. Flow at 100 gpm—brick and fence present (388 ft from inlet—downstream).

Figure 15. Flow at 100 gpm—brick and fence present (688 ft from inlet—upstream).



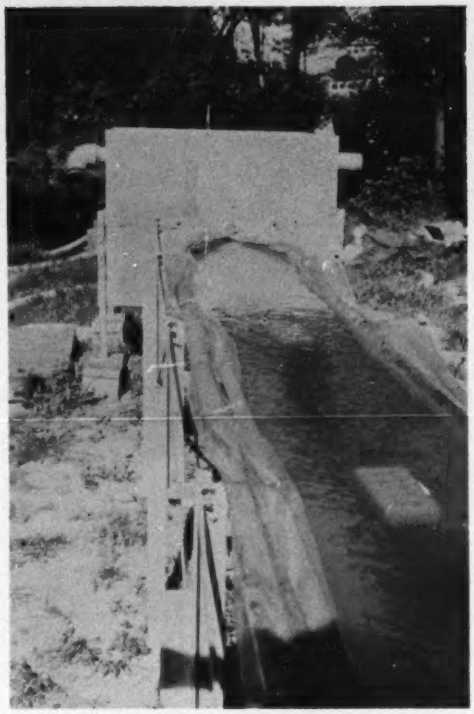


Figure 18. Flow at 150 gpm—brick and fence present (20 ft from inlet—upstream).

Figure 19. Flow at 150 gpm—brick and fence present (324 ft from inlet—upstream).

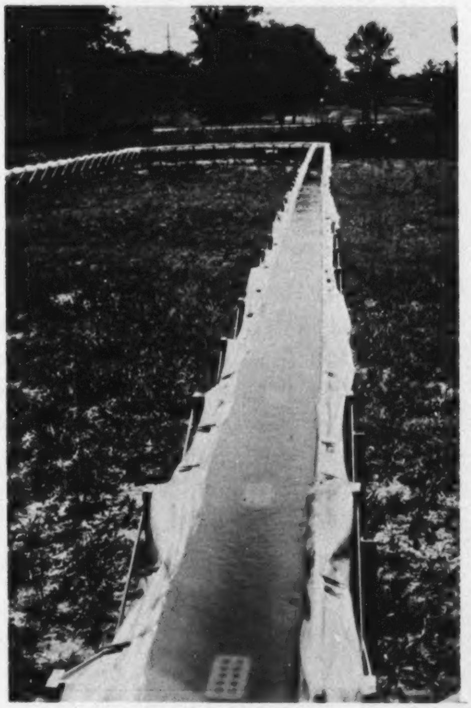


TABLE 1

Run	Avg. Width (ft)	Channel Conditions	Run	Avg. Width (ft)	Channel Conditions
1	1.02	A	18	1.231	C
2	1.07	A	19	1.256	C
3	1.05	A	20	1.270	C
4	1.06	A	21	1.305	C
5	1.12	A	22	1.192	D
6	1.15	A	23	1.225	D
7	1.16	A	24	1.252	D
8	1.15	A	25	1.272	D
9	1.142	B	26	1.322	D
10	1.16	B	27	1.278	E
11	1.20	B	28	1.324	E
12	1.229	B	29	1.107	F
13	1.242	B	30	1.178	F
14	1.261	B	31	1.214	F
15	1.160	C	32	1.236	G
16	1.182	C	33	1.281	G
17	1.216	C	34	1.309	H

DESIGNATIONS OF CHANNEL CONDITIONS

A - Channel without obstructions.
B - Channel with rolls of chicken fencing present.
C - Condition B plus brick laid length-wise on fence, the broad side up.
D - Condition B plus brick laid width-wise on fence, the broad side up.
E - Condition B plus brick laid width-wise on fence, the narrow side up.
F - Condition C without fence.
G - Condition D without fence.
H - Condition E without fence.

TABLE 2

							Dissolved Oxygen Pick-up MG/liter/sq. ft surface area							
Run	Date	Flow (gpm)	Avg. Depth (°C)	Avg. Depth (ft)	Avg. Velocity (ft/sec)	Time of Passage for 668' (min-sec)	Avg. Def.	Pick up	Avg. Def.	Pick up	Avg. Def.	Pick up	Avg. Def.	Pick up
1	6-19-67	128	26.1	0.429	0.938	11-0	6.72	.0011	5.69	.0015	4.57	.0014	4.92	.0010
2	6-27-67	85	27.3	0.346	0.880	12-15	6.61	.0016	5.96	.0009	5.66	.0011	5.09	.0008
3	6-26-67	88	27.2	0.327	0.995	12-30	6.62	.0011	6.20	.0012	5.27	.0008	4.27	.0007
4	6-29-67	100	23.0	0.350	1.014	11-30	6.53	.0011	3.78	.0010	5.26	.0008	4.76	.0004
5	6-29-67	150	24.6	0.402	1.193	9-45	6.43	.0005	5.86	.0004	5.06	-.0008	5.03	.0004
6	7- 1-67	175	30.4	0.438	1.223	9-20	4.64	.0002	4.33	.0003	4.21	.0002		
7	6-30-67	200	29.4	0.462	1.297	9-0	5.63	.0008						
8	7- 1-67	200	28.0	0.458	1.312	9-0	6.41	.0008	6.03	.0004	5.65	.0006		
9	7- 8-67	75	29.8	0.440	0.525	21-30	4.42	.0008	4.09	.0007				
10	7- 5-67	100	24.6	0.467	0.634	19-0	4.62	.0005	4.75	.0006	4.58	.0002		
11	7- 6-67	125	28.0	0.503	0.708	18-45	5.09	.0005	3.31	-.0003	4.20	.0009	5.56	.0002
12	7- 6-67	150	27.9	0.562	0.720	16-45	5.37	.0012	4.37	.0014	3.85	.0007		
13	7- 8-67	175	27.1	0.576	0.816	15-30	3.67	.0007	3.67	.0005	3.56	.0003	4.45	.0005
14	7- 8-67	200	30.6	0.611	0.843	14-0	4.30	.0004	4.04	.0004	3.75	.0005	4.82	.0011
15	7-19-67	75	25.7	0.456	0.496	24-0	3.92	.0006	4.13	.0008	2.30	-.0006		
16	7-19-67	100	28.6	0.484	0.603	21-0	4.57	.0007	3.87	.0007	2.80	.0002		
17	7-19-67	125	27.1	0.528	0.663	18-30	5.11	.0007	5.96	.0008	3.89	.0002		

Run	Date	Flow (gpm)	Avg. Depth (°C)	Avg. Depth (ft)	Avg. Velocity (ft/sec)	Time of Passage for 668' (min-sec)	Dissolved Oxygen Pick-up MG/liter/sq. ft surface area							
							Avg. Def.	Pick up	Avg. Def.	Pick up	Avg. Def.	Pick up	Avg. Def.	Pick up
18	7-20-67	150	24.7	0.573	0.696	17-30	5.64	.0008	4.97	.0006	3.55	.0002		
19	7-12-67	175	28.5	0.609	0.744	16-0	5.78	.0007	4.86	.0003	3.99	.0003		
20	7-12-67	200	29.5	0.623	0.804	15-15	4.80	.0005	3.55	.0002	2.53	.0000		
21	7-20-67	250	30.8	0.694	0.876	13-5	4.59	.0003	4.29	.0005	4.87	.0005	4.30	.0005
22	7-13-67	75	28.6	0.504	0.423	28-0	6.21	.0014	5.00	.0011	3.76	.0007		
23	7-13-67	100	21.9	0.568	0.474	25-30	5.29	.0008	4.19	.0006	5.95	.0015		
24	7-13-67	125	18.2	0.608	0.534	22-30	5.41	.0006	4.23	.0008				
25	7-13-67	150	28.4	0.636	0.597	20-30	6.02	.0010	5.44	.0008	4.58	.0005		
26	7-12-67	175	27.5	0.699	0.640	17-30	6.32	.0004	5.40	.0005	3.99	.0003		
27	7-13-67	125	19.0	0.642	0.490	25-0	4.84	.0005	4.47	.0003	7.68	.0010		
28	7-14-67	175	18.7	0.692	0.617	21-0	3.80	.0002	3.67	.0002	3.76	.0002		
29	4-21-67	75	29.7	0.410	0.586	20-0	5.94	.0018	3.90	.0007				
30	7-21-67	125	29.8	0.490	0.738	15-45	5.22	.0014	5.29	.0009	4.66	.0006	3.84	.0006
31	7-24-67	175	29.7	0.555	0.855	13-45	5.44	.0007	4.95	.0008	4.37	.0006		
32	7-25-67	125	29.1	0.574	0.581	19-30	5.11	.0008	5.80	.0012	3.71	.0008		
33	7-24-67	175	31.0	0.655	0.664	17-30	5.08	.0007	5.17	.0014	3.92	.0004		
34	7-25-67	175	30.7	0.703	0.602	19-15	5.86	.0001	5.34	.0004	4.61	.0004		

The two methods that will be used to evaluate (a) are the shear velocity, $\overset{*}{U}$, and the Manning's roughness coefficient, n.

$$\overset{*}{U} = gRS$$

with

g = gravity constant
R = hydraulic radius
S = energy slope

$$n = \frac{1.486}{V} R^{2/3} S^{1/2}$$

with

V = average longitudinal velocity

The effect of (b) will be evaluated by using the longitudinal mixing coefficient, D_L. To incorporate the use of D_L, a flow-through curve will be used of concentration vs. time. In determining D_L from the flow-through curve, the availability of a digital computer will allow the use of the dispersion model:

$$\frac{\partial c}{\partial t} = D_L \frac{\partial^2 c}{\partial x^2} - \overset{*}{U} \frac{\partial c}{\partial x}$$

and will be evaluated by a least squares method. Other methods will also be considered, especially Hay's$^{(30)}$ model since it allows for possible storage of the tracer behind the bricks. Since it is impossible to hold the several parameters constant, and then vary only one, it will be necessary to incorporate a statistical regression analysis to determine the accuracy of the various resulting equations.

Utilizing the collected data, a method of predicting D_L will be formulated and finally a method of predicting k_2 from the basic hydraulic data will be attempted.

PHOTOSYNTHETIC OXYGEN PRODUCTION

Included in Table 3 is a list of 29 experiments that were carried out in the latter part of the summer and fall of 1967. This table indicates the flow, time of passage, the average dissolved oxygen (D.O.) deficit, average water temperature, average D.O. pick-up per hour, average net D.O. per hour, and the average saturation values. The net D.O. per hour was determined by subtracting the physical reaeration from that of the total amount of oxygen increase over a certain time period between the beginning and last station. Physical reaeration values were picked from those experiments that were carried out under similar

TABLE 3

PHOTOSYNTHETIC OXYGEN PRODUCTION

Run	Date	Flow gal/min	Avg. Temp. °C	Sat. Value	Avg. D.O. Deficit	Avg. D.O. Pick-up	Avg. D.O. ppm/hr	Reaer. ppm/hr	Net D.O. ppm/hr	Time of Pass. min
1	7-28-67	75	27.9	7.9	5.3	.90	4.6	2.6	2.0	11'48
2	7-29-67	75	23.3	8.65	5.3	.90	4.6	2.6	2.0	11'48
3	8- 1-67	100	26.8	8.10	5.2	.80	4.2	2.6	1.6	11'30
4	8- 2-67	100	25.9	8.2	5.4	.75	3.9			11'30
5	8- 3-67	150	28.9	7.8	5.9	.70	4.25	1.85	1.4	9'50
6	8- 4-67	150	25.9	8.2	5.6	.80	4.85	1.85	3.0	9'50
7	8- 8-67	200	26.9	8.1	4.8	.55	6.0			8'56
8	8- 9-67	200	29.3	7.7	6.0	.90	6.5	2.05	4.45	8'56
9	8-11-67	200	19.2	9.4	6.05	.85	3.67			8'56
10	8-15-67	100	25.1	8.4	5.9	1.15	6.0			11'30
11	8-16-67	100	25.9	8.2	6.5	1.25	5.7			11'30
12*	8-18-67	75	24.5	8.4	.70	- .05	- .15			20'15
13*	8-19-67	75	21.3	9.0	.15	- .25	- .75			20'15
14*	8-21-67	75	20.0	9.2	.4	- .05	- .15			20'15
15*	8-22-67	75	20.7	9.1	- .05	- .15	- .45			20'15
16*	8-23-67	125	21.8	8.8	- .20	- .05	- .20			15'45
17*	8-24-67	125	21.7	8.9	- .20	- .10	- .40			15'45
18*	8-25-67	125	27.4	8.0	7.45	1.50	5.70	1.6	5.1	15'45
19*	8-26-67	125	21.9	8.8	3.3	1.15	4.4	1.6	2.8	15'45
20*	8-31-67	150	20.1	9.2	7.8	1.05	4.4			14'15
21*	9- 1-67	150	17.9	9.5	5.2	.80	3.4			14'15
22*	9- 4-67	150	21.2	9.0	.45	.15	.65			14'15
23*	9- 5-67	150	24.5	8.45	.40	.25	.85			14'15
24**	9-11-67	75	15.5	10.1	.25	- .05	- .10			33'45
25** }	9-12-67	75	17.6	9.6	6.6	1.25	2.2			33'45
26** }	9-13-67	75	15.4	10.1	6.05	- .05	1.4			33'45
27	9-17-67	150	20.8	9.0	5.35	.75	4.6			9'45
28 }	9-18-67	150	20.4	9.1	5.5	.95	1.70			9'45
29 }	9-19-67	150	21.4	8.9	5.1	.40	2.50			9'45

* Bricks positioned longways in the channel.

** Bricks positioned crossways in the channel.

} Indicates day and night runs under similar conditions.

conditions except that the system was poisoned to destroy photosynthetic, bacterial, and community respiratory activity. As indicated earlier, the contents of Table 3 are the average of two to eight samples that were secured over a period of one day. As a rule, samples were taken nearly every hour for dissolved oxygen. Daily samples for light and dark bottle, suspended and attached organisms, and 5 day biochemical oxygen demand (B.O.D.) were also secured. Below is a list of the more common genera of organism that were encountered during the sampling period.

Anabaena circinalis
Ankistrodesmus sp.
Aphanizomenon sp.
Asterionella formosa
Ceratium hidundinella
Chlorella sp.
Chlamydomonas sp.
Closterium sp.
Cocconeis sp.
Cosmarium
Cryptomonas ovata
Cyclotella spp.
Cymbella sp.
Cymatopleura sp.
Diatoma vulgare
Difflugia sp.
Euglena fusca
Euglena pisciformis
Euglena viridis
Dinobryon sertularia
Desmidium sp.
Fragilaria crotonensis
Gomphosphaeria aponina
Keratella sp.
Lyngbya sp.
Mallonomas sp.
Melosira granulata
Melosira varians
Microcystis aeruginosa
Navicula spp.
Nitzschia sp.
Oscillatoria sp.
Pediastrum simplex
Phacotus lenticularis
Phacus stokesii
Pleurosigma sp.
Rotifera, unidentified
Scenedesmus sp.
Stephanodiscus sp.
Stauroneis sp.
Surirella sp.
Synedra acus
Synedra capitata
Tabellario fenestrata

FUTURE EXPERIMENTATION

ATMOSPHERIC REAERATION

Several changes are anticipated for the summer of 1968, the primary one being the proposed continuous feed of Na_2SO_3 for prolonged periods of time to allow for several samples to be obtained at the same dissolved oxygen deficit. This seems to indicate that fewer runs will be made in 1968 but of longer duration. A possible solution would be the use of two or three crews to provide a continuous operation during the few favorable summer months.

The use of an oxygen depleted flow-through curve will continue in 1968, but also Rhodamine WT dye and possibly other tracers will be used in conjunction with a fluorometer to measure concentrations. Several curves will be obtained for each flow to insure reliability.

The solids content will be much more closely controlled with a centrifugal filter attached to the storage reservoir. The runs at various channel conditions will also incorporate an overlap of temperatures so that mutual temperatures will be available for all runs; this may require the use of some temperature controlling mechanism during July and August.

A new polyvinyl liner will be installed in the spring to provide a more exact fit with the cross-sectional shape.

An expansion of the experimental runs from the channel to a well-defined small stream may be attempted later in the summer of 1968.

PHOTOSYNTHETIC OXYGEN PRODUCTION

The experimental channel will be broken into two equal segments and each of these in turn will be divided longitudinally into two parts, resulting in a total of four independent channels.

When a number of variables, like light, velocity, dissolved oxygen deficit, and population types, are to be compared in a natural environment, it is obvious that the comparison should be carried out under conditions which are as nearly alike as practicable, because any extraneous variation would add to the experimental error. It is for this reason, that a confounded factorial design—in which unimportant comparisons are deliberately confused for the purpose of assessing the more important comparisons with greater precision—will be utilized in this research problem.

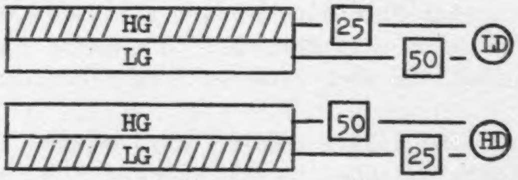
The four variables, which play the major role in photosynthetic oxygen production, namely light, current, the aquatic community, and dissolved oxygen deficit will be evaluated.

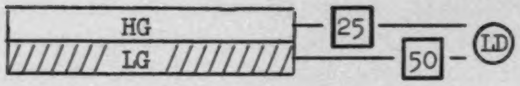
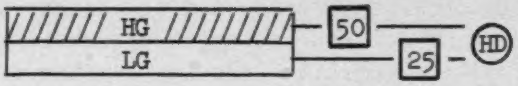
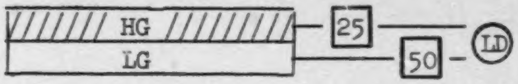
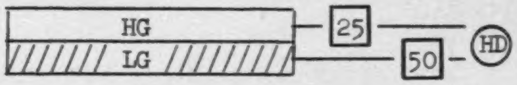
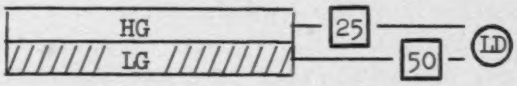
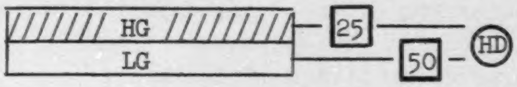
Two 7,000 gal storage reservoirs, four flow meters, two outlet and inlet boxes, and four channels will be utilized in this factorial design to compare and evaluate what effect two levels of each variable has on the photosynthetic activity by periphyton and phytoplankton. The variables are:

Light	Optimum (H.L.)	Shaded (L.L.)
Flow	50 gal/min (H.F.)	25 gal/min (L.F.)
Growth	Periphyton and phytoplankton (H.G.)	Phytoplankton (L.G.)
Oxygen deficit	High deficit (H.D.)	Low deficit (L.D.)

By setting up the following factorial design the variables and their interactions will be evaluated.

No. 1	(a)LL	LF	LG	HD	(b)
	(b)LL	LF	HG	LD	(c)
	(c)HL	HF	LG	LD	(d)
	(d)HL	HF	HG	HD	(a)



No. 2	(a)LL	HF	LG	LD	(d)	
	(b)LL	HF	HG	HD	(a)	
	(c)HL	LF	LG	HD	(b)	
	(d)HL	LF	HG	LD	(c)	
No. 3	(a)LL	HF	LG	HD	(b)	
	(b)LL	HF	HG	LD	(c)	
	(c)HL	LF	LG	LD	(d)	
	(d)HL	LF	HG	HD	(a)	
No. 4	(a)LL	LF	LG	LD	(d)	
	(b)LL	LF	HG	HD	(a)	
	(c)HL	HF	LG	HD	(b)	
	(d)HL	HF	HG	LD	(c)	

Light

It is clear that algal photosynthesis and hence oxygen production is dependent on light. Variation in total radiation with depth is probably of relatively minor importance in a small stream, but in a large stream bearing a heavy silt or organic waste load, turbidity can reduce light penetration to a point which curtails or completely prevents plant growth, including that of phytoplankton. For this reason, two of the four channels during each experimental run will be covered to reduce the solar radiation comparable to that of a turbid stream, while the remaining two channels will be open to direct sunlight. Measurement of the incident and submerged illumination both in the shaded and uncovered channel will be determined by light integrating units described by Wojtalik, Ball, and Hooper.(31)

Flow

In moving water, velocity plays an important factor in biomass production. It was pointed out as early as 1926 by Ruttner who partially explained the "inherent current demand" phenomenon. He pointed out that in quiet water a film deficient in vital materials forms at the surface of a plant or animal, and in swift water this film is swept away. Moving water, he said, "is not absolutely, but rather physiologically, richer in oxygen and nutrients." This "physiological richness," according to Whitford (1960), can be explained on the basis of the laws of cohesion and diffusion. In still water nutrients diffusing inward are lowest in concentration at the cell surface and increase in concentration with increase in the distance from the cell to a point where full concentration is reached. In swift water the distance between full concentration and the cell surface is reduced, increasing the diffusion rate. This increases the exchange of nutrients between cell and the surrounding water. According to Whitford the current velocity had to exceed .5 ft (15 cm) per second to eliminate the area of low concentration of nutrients "diffusion shell" between the point of full concentration and the surface of the cell. To compare and evaluate this phenomenon, flows of 50 gal/min and 25 gal/min with respective velocities of 1.0 ft/sec and .45 ft/sec are chosen.

Growth

Periphyton and phytoplankton play an important role in flowing waters. Because of their ubiquitousness and rapid turnover, they provide both food and shelter for the benthic fauna of a stream. Since the organisms involved are not equipped with a means of procuring the essential elements from the stream bed, the production of these communities is also closely related to the characteristics of the water mass flowing by. Consequently, an evaluation of the periphyton and phytoplankton community has long been recognized as a means of evaluating stream biodynamics.

In this research, the suspended fauna of the Huron River will be designated as the "phytoplankton community." Given adequate time, nutrients, and other favorable environmental factors, certain of these organisms will attach, colonize, and eventually become a periphyton community. One of the objectives of this research is to determine the oxygen production, oxygen utilization, and possible interactions with other variables of each of these communities. The following procedures will be used to establish these communities.

In two of the four channels, the phytoplankton-periphyton community will be permitted to reach maximum growth or equilibrium at a flow of 25 gal/min through the process of recirculation from the reservoirs which are replenished daily with river water. Maximum growth or equilibrium, a situation where the number of cells being sloughed off from the attached community are equal to the new cells formed, will be determined through phyto-pigment concentration and total cell counts. The water depth and therefore the attached growth in the channels will remain nearly the same throughout the experimental period by raising the overflow weir at low flows. After establishment of this attached community, the experimental runs will start.

Dissolved Oxygen Deficit

The level of dissolved oxygen in a stream is dependent on many diverse factors. Streams issuing from springs tend to be low in dissolved oxygen, but usually the upper course of streams in temperate regions is well oxygenated. Impoundment of the stream or retardation of its flow, as in the lower course of most streams where deposition of bottom sediments occur, may likewise serve to depress the dissolved oxygen. The entry of an important sewage outfall, other things being equal, will tend to depress the dissolved oxygen levels.

Besides physical reaeration, it is felt that photosynthetic oxygen production, in the presence of a rich phytoplankton fauna, is a great asset. These organisms, provided solar radiation is not limiting, will float through a reach of high oxygen deficit, continuously producing photosynthetic oxygen.

According to previous data, the oxygen production by phytoplankton, under artificially produced oxygen deficits, was twice that of physical reaeration, and varied with the deficits. It is for this reason that further experimentation with a high and low oxygen deficit in conjunction with other variables will be investigated. The dissolved oxygen in the reservoirs will be depressed to a desired level with the addition of sodium sulfite in the presence of a cobalt catalyst. Physical reaeration will be separately evaluated under the selected oxygen deficits and the experimental flow conditions with all organisms killed. This can then be subtracted from total oxygen pickup for determination of photosynthetic oxygen contribution.

Typical Experimental Run

The following is a description of the methods and procedures that would be carried out during a typical experimental run, after the periphyton has built up to maximum growth in two of the four channels.

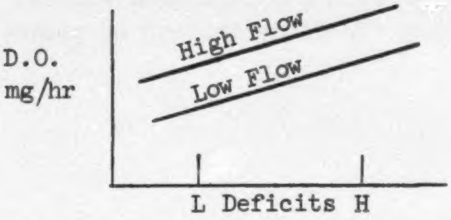
The two 7,000 gal capacity reservoirs are filled, by means of four 110 gal/min capacity pumps, with water from the Huron River. During this process, water samples are secured from each of the reservoirs for chlorophyll pigment analysis, cell enumeration and identification. In addition, a Turner Model 111 Fluorometer, that has been modified as suggested by Baily,(32) will be utilized to measure and record chlorophyll concentrations of the incoming water. After filling, each of the reservoirs will be treated with sodium sulfite in the presence of cobalt catalyst to produce the desired oxygen deficit level. After a uniform D.O. level is reached in each of the reservoirs, the water is then pumped to the channels through flow meters. During the period of five to ten minutes, the time necessary to receive constant flow through the channel, light and dark bottle samples will be taken from each of the reservoirs. The reservoirs then will be completely covered to minimize photosynthetic activity, and through this procedure a fairly constant oxygen deficit level can be maintained. In the meantime the flow will become constant through each of the four channels and the time of passage through each of the channels will be rechecked. The maximum pumping capacity out of each storage reservoir is only 75 gal/min and therefore a period of 80 min is available to carry out the experiment. The actual time of an experimental run will be one hour. The remaining 20 min of the flow period will be utilized to establish constant flow conditions and to determine if any population changes have occurred during the experiment.

During the experimental run, samples for dissolved oxygen will be taken at each end of each of the channels every ten minutes. In addition, continuous recording devices for dissolved oxygen (Beckman Oxygen Analyzer), air and water temperature (Weahter Hawk Thermometer), solar radiation, and pH will be utilized. Additional light and dark bottle samples from the effluent will be secured to determine productivity. Several night runs will be carried out in conjunction with day runs to evaluate community respiration and atmospheric reaeration.

Expected Results

The anticipated data will probably be presented and analyzed by the following method:

GRAPHICAL

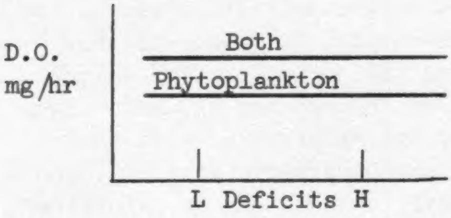


STATISTICAL

Main effect flow
Main effect deficit
Interaction of flow and deficit

Objective: 1. Determine if higher deficit leads to increased rate or reaeration.
2. Determine if higher flow increases reaeration and if so in the same manner over both deficits. (interaction)

GRAPHICAL

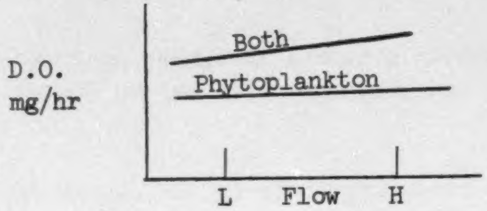


STATISTICAL

Main effect
Growth interaction of deficit and growth

Objective: 1. Determination of D.O. produced by population.
2. Do deficits cause changes in D.O. produced?
3. Are the slopes with deficits the same for both population?

GRAPHICAL

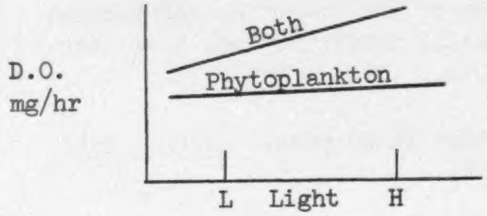


STATISTICAL

Interaction of flow and populations.

1. Determine if flow effects D.O. production differently with population, at both deficits.

GRAPHICAL



STATISTICAL

Main effect of light
Interaction of light and population

1. Determine the effects of light and at what point does light become limiting for each of the populations.

This is a short presentation of the type of data that will be collected, analyzed, and illustrated. It is understood that some of the variables and their interaction are more important than others, and it is expected that this will become more obvious at the end of the first year's activity.

SUMMARY AND CONCLUSIONS

The experimental channel has been equipped to investigate the phenomenon of atmospheric reaeration and the photosynthetic oxygen production processes. The experimental data collected in 1967 is being analyzed and generally indicates:

(a) Sufficient variability of flow ranges and channel conditions can be obtained for the proposed study.

(b) A very high degree of control over the variables can be achieved.

(c) Flow-through curves are significantly affected by the various channel conditions.

(d) Oxygen pick-ups of 1.0 ppm or more can be obtained at deficits of 7.0 to 8.0 ppm.

(e) Time of passage from station A to station D can be in excess of 30 min allowing ample time for atmospheric reaeration and photosynthetic oxygen contribution.

(f) The effect of the presence of oxygen-producing biota in the system can be determined.

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SECTION VI

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